

See discussions, stats, and author profiles for this publication at: <https://www.researchgate.net/publication/268691211>

Diversity of peptide toxins in stinging ant venoms

Article in *Toxicon* · October 2014

DOI: 10.1016/j.toxicon.2014.10.021

CITATIONS
92

READS
1,286

7 authors, including:



Samira Aili

University of Technology Sydney

13 PUBLICATIONS 416 CITATIONS

[SEE PROFILE](#)



Axel Touchard

French National Institute for Agriculture, Food, and Environment (INRAE)

33 PUBLICATIONS 587 CITATIONS

[SEE PROFILE](#)



Matthew P Padula

University of Technology Sydney

198 PUBLICATIONS 3,187 CITATIONS

[SEE PROFILE](#)



Jerome Orivel

French National Centre for Scientific Research

312 PUBLICATIONS 5,589 CITATIONS

[SEE PROFILE](#)

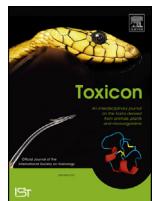
Some of the authors of this publication are also working on these related projects:



Ant garden [View project](#)



The aquatic macrofauna of tank bromeliads [View project](#)



Review

Diversity of peptide toxins from stinging ant venoms



Samira R. Aili ^{a,1}, Axel Touchard ^{b,1}, Pierre Escoubas ^c, Matthew P. Padula ^a,
Jérôme Orivel ^b, Alain Dejean ^{b,d,**}, Graham M. Nicholson ^{a,*}

^a Neurotoxin Research Group, School of Medical & Molecular Biosciences, University of Technology Sydney, NSW 2007, Australia

^b CNRS, UMR Écologie des Forêts de Guyane (EcoFoG), Campus Agronomique, BP 316, 97379 Kourou Cedex, France

^c VenomeTech, 473 Route des Dolines – Villa 3, 06560 Valbonne, France

^d Laboratoire Écologie Fonctionnelle et Environnement, Université de Toulouse, 118 Route de Narbonne, 31062 Toulouse, France

ARTICLE INFO

Article history:

Received 3 September 2014

Accepted 27 October 2014

Available online 28 October 2014

Keywords:

Ant venom

Peptides

Venom biochemistry

Disulfide linkage

Chemotaxonomy

ABSTRACT

Ants (Hymenoptera: Formicidae) represent a taxonomically diverse group of arthropods comprising nearly 13,000 extant species. Sixteen ant subfamilies have individuals that possess a stinger and use their venom for purposes such as a defence against predators, competitors and microbial pathogens, for predation, as well as for social communication. They exhibit a range of activities including antimicrobial, haemolytic, cytolytic, paralytic, insecticidal and pain-producing pharmacologies. While ant venoms are known to be rich in alkaloids and hydrocarbons, ant venoms rich in peptides are becoming more common, yet remain understudied. Recent advances in mass spectrometry techniques have begun to reveal the true complexity of ant venom peptide composition. In the few venoms explored thus far, most peptide toxins appear to occur as small polycationic linear toxins, with antibacterial properties and insecticidal activity. Unlike other venomous animals, a number of ant venoms also contain a range of homodimeric and heterodimeric peptides with one or two interchain disulfide bonds possessing pore-forming, allergenic and paralytic actions. However, ant venoms seem to have only a small number of monomeric disulfide-linked peptides. The present review details the structure and pharmacology of known ant venom peptide toxins and their potential as a source of novel bioinsecticides and therapeutic agents.

© 2014 Elsevier Ltd. All rights reserved.

1. Stinging ant biodiversity

Hymenopterans are among the most speciose group of venomous animals. With approximately 120,000 currently described species (van Emden, 2013), they are significantly more diverse than the major venomous phyla including spiders (44,906 species), snakes (3496 species), cone snails (3253 species), sea anemones (3248 species) and scorpions (1454 species) (Fautin, 2014; Hallan, 2005; Kohn and Anderson, 2009; Platnick, 2014; Uetz and Hošek, 2014). Among the stinging aculeate Hymenoptera, ants and wasps (superfamily Vespoidea) and bees together with sphecoid wasps (superfamily Apoidea) are sister groups (Johnson et al., 2013). Ants (family Formicidae) evolved from wasp-

like ancestors between 115 and 135 million years ago (Brady et al., 2006) and became a diverse taxonomical group with ~13,000 extant species belonging to 21 subfamilies (Agosti and Johnson, 2005; AntWeb, 2014). Due to their ubiquitous nature in terrestrial environments, and the fact that they constitute 15–20% of the animal biomass in tropical rainforests (Hölldobler and Wilson, 1990; Wilson, 1990), ants are arguably amongst the most abundant venomous animals.

Ants that belong to the subfamilies Formicinae, Dolichoderinae, Aneuretinae and Dorylinae lost their ability to sting during evolution (Fig. 1). Instead, they usually spray their venoms or have a residual, but non-functional, abdominal stinger. Also, it is unclear if the recently discovered subfamily Aenictogitoninae is venomous or not, as only male castes have been seen and females (workers and queens) are yet to be described (Brady et al., 2006). The remaining 16 subfamilies are all stinging ants (Fig. 1) and comprise of ~9100 extant species. This makes ants taxonomically more diverse than scorpions, snakes and cone snails. However, this biodiversity is not equally distributed within stinging ant subfamilies (Figs. 1 and 2). For example, Myrmicinae is the most speciose ant subfamily, with

* Corresponding author.

** Corresponding author. Écologie des Forêts de Guyane, Campus Agronomique, BP 316, 97379 Kourou Cedex, France.

E-mail addresses: alain.dejean@wanadoo.fr (A. Dejean), [G.M. Nicholson](mailto:Graham.Nicholson@uts.edu.au).

¹ Authors contributed equally to this review.

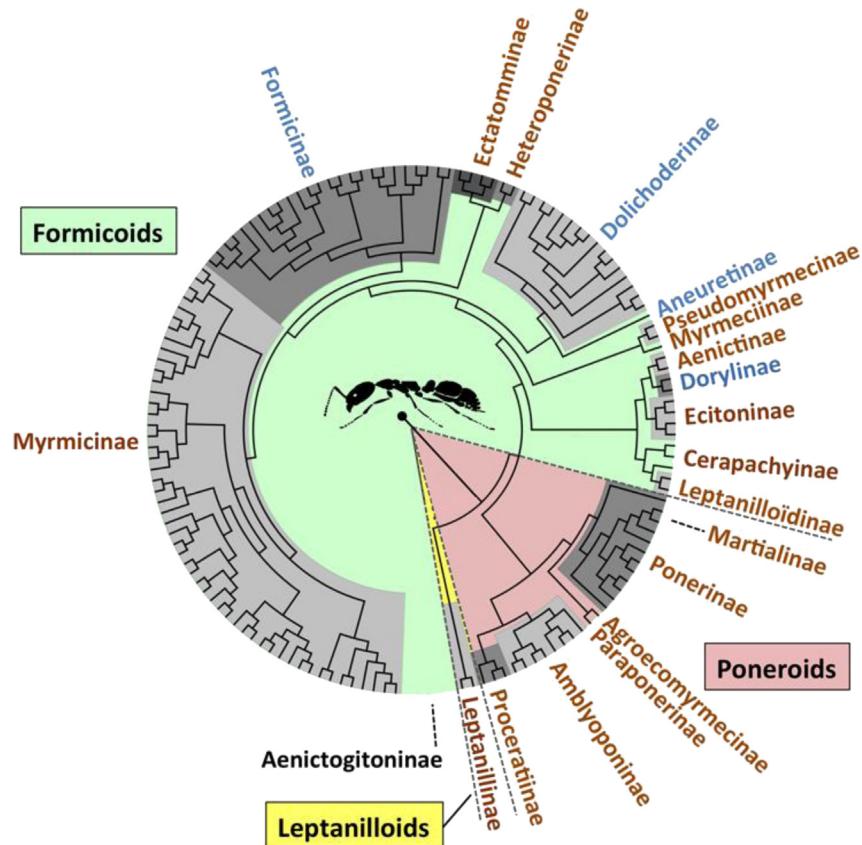


Fig. 1. Ant subfamily relationships as inferred from molecular phylogenetic studies. Phylogenetic relationships were generated from the S1573 TreeBASE data file (Moreau et al., 2006) using the FigTree v1.4.2 software package (<http://tree.bio.ed.ac.uk/software/figtree/>). Phylogenetic relationships for the subfamilies Aenictogitoninae and Martialinae are currently unavailable. During evolution, four subfamilies lost their capacity to sting (blue text). Remaining subfamilies represent stinging ants (brown text). Ant clades are shaded green (Formicoids), red (Poneroids) and yellow (Leptanilloids). Females of subfamily Aenictogitoninae (black text) remain undiscovered and so this subfamily cannot be classified as either stinging or non-stinging. For clarification of colours in this figure, refer to the web version of this article. Note added in proof: Recently, the ant subfamilies Leptanilloidinae, Cerapachyinae, Ecitoninae, Dorylinae, Aenictinae and Aenictogitoninae have been regrouped into one subfamily; Dorylinae (Brady, S., Fisher, B., Schultz, T., Ward, P., 2014. The rise of army ants and their relatives: diversification of specialized predatory doryline ants. BMC Evol. Biol. 14, 93–106.).

~6500 extant species, with a widespread distribution throughout the world. However, ponerine ants that belong to the subfamily with the second highest number of ants, Ponerinae (~1200 species), are mainly confined to tropical rainforests (AntWeb, 2014; Johnson et al., 2013). Furthermore, the subfamilies Paraponerinae and Martialinae only contain a single ant species both of which are found in Neotropical areas. Thus, taxonomic diversity varies within each ant subfamily however there is little doubt that ant venoms likely constitute a vast source of unique bioactive toxins.

2. Ant venom functions

Ant venom is composed of a complex mixture of chemicals such as proteins, enzymes, biogenic amines, peptides, hydrocarbons, formic acid and alkaloids (Davies et al., 2004; Kem et al., 2004; Yi et al., 2003). All these compounds are produced by the venom gland, which consists of two free cylindrical elongated and convoluted tubes, linked to a venom reservoir (Ortiz and Mathias, 2006). The venom secreted by the tubular glands is stored in the reservoir, linked to the delivery apparatus and, for example, can deliver up to 130 µg of venom after each sting (Schmidt, 1990). The stinger itself is a modified ovipositor located at the distal base of the abdomen. Ants use their venom for several purposes such as a defence against predators/competitors and microbial pathogens, for predation, as well as for social communication (Orivel et al., 2001; Schmidt, 1982). Hence, ant venoms have evolved to carry out many different functions.

2.1. Offensivevenoms

Ants are one of the leading predators of invertebrates in most ecosystems (Brady et al., 2006). They have developed, through natural selection, a vast arsenal of behavioural adaptations and weapons to subdue their prey including trap-mandibles and potent venoms (Casewell et al., 2013). Ant venom has paralytic and lethal effects on many arthropods (Maschwitz et al., 1979; Orivel and Dejean, 2001) and many ants are generalist predators, preying on numerous classes of invertebrates. Nevertheless, many ants are specialised predators and only feed on a restricted group of species. Such specialised hunters prey exclusively on earthworms, isopods, centipedes, millipedes, polyxena, collembolan, termites, other ants or even spider eggs (Cerdá and Dejean, 2011). Solitary hunting is the most common hunting behaviour employed by primitive ants such as ponerines. However, many ants have also developed a cooperative hunting behaviour such as army ants exhibiting extreme group hunting behaviour.

The ecological diversity of ants is also revealed in their preference for various nesting habitats. Predatory ants are primarily ground, or litter-dwelling, predators. However, some ants have evolved predatory behaviours adapted to foraging in trees (arboreal ants) and exhibit adaptions to prevent their prey from escaping by flying away, jumping or dropping. Accordingly, venoms of solitary-foraging, arboreal predatory ants are believed to be more efficient than ground-dwelling species at rapidly immobilising prey (Orivel and Dejean, 2001). Thus, the use of venom as an offensive weapon

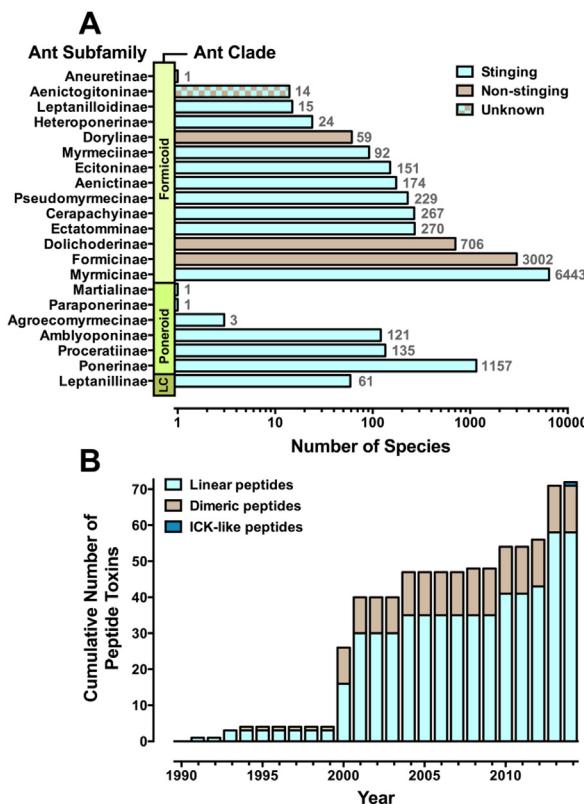


Fig. 2. (A) Species richness of ant subfamilies. Ants have been grouped according to three clades, where LC represents the single genus Leptanilloid clade. Stinging ants are represented by cyan bars and comprise around 70% of all ant species. Non-stinging ant subfamilies are depicted by brown bars. The total number of species in each subfamily is noted at right of each bar. The Aenictogitoninae subfamily is currently unclassified. Note added in proof: Recently, the ant subfamilies Leptanilloidinae, Cerapachyinae, Ectoninae, Dorylinae, Aenictinae and Aenictogitoninae have been regrouped into one subfamily: Dorylinae (Brady, S., Fisher, B., Schultz, T., Ward, P., 2014). The rise of army ants and their relatives: diversification of specialized predatory doryline ants. BMC Evol. Biol. 14, 93–106.). (B) Cumulative total number of peptide-toxin sequences reported from ant venom studies since the first described venom peptide (poneratoxin) in 1991, showing the three main structural classes: cyan, linear peptides; brown, dimeric peptides; teal, ICK-like peptides. Ant venom peptides remain barely investigated with only 72 peptides sequenced to date. For clarification of colours in this figure, refer to the web version of this article.

is likely to be the major driver of the venom composition during evolution. This has been shown with the differing composition and toxicity of venoms from arboreal *versus* ground-dwelling species of *Pseudomyrmex* and *Pachycondyla* (Dejean et al., 2014; Orivel and Dejean, 2001; Touchard et al., 2014b). The wide ranging diet and hunting behaviours of ants are therefore likely to drive major differences among ant venom toxins.

2.2. Defensive venoms

Eusociality within hymenopteran colonies offers a range of evolutionary advantages including the capability of mounting a collective defence against vertebrate and other arthropod predators, the ability to gather and store food and nutrients more efficiently, and to specialize in specific tasks, such as to care cooperatively for offspring (Wilson, 1971). Nevertheless, these benefits can only be realized if the colony can defend against large predators who find the large biomass of the colony a potential food source worth their effort, in contrast to preying upon solitary hymenopterans. The evolution of venom in hymenopterans therefore provided a mechanism of defence against large intelligent vertebrate predators and enabled them to develop complex

societies. The combination of algic and lethal actions of ant venom is therefore thought to be critical in the long term evolutionary success of insect stings to deter large predators (Schmidt, 2014). For example, some ant stings are known to be extremely painful for humans. These include stings by fire ants (*Solenopsis* spp.), ponerine ants (*Pachycondyla* spp.) or the bullet ant (*Paraponera clavata*). In particular, bullet ants have been classified as producing the most painful sting among all hymenoptera and the third most painful sting of all venomous animals (Schmidt et al., 1983; Starr, 1985).

It is also clear that some ants, such as the *Pogonomyrmex* group of harvester ants, have developed venoms primarily for defence against vertebrates (Schmidt and Snelling, 2009). For example, the venom of *Pogonomyrmex badius* is highly toxic towards mice, but not very toxic towards insects. Therefore, *Pogonomyrmex* ants do not appear to employ their venom to hunt, but use it exclusively as a deterrent against vertebrate predators (Schmidt and Blum, 1978a, 1978b), akin to the defensive role of bee venom against vertebrates. Some *Pseudomyrmecinae* ants have also evolved a ‘defensive venom’ as part of a mutualistic relationship with myrmecophytes. Myrmecophytes are plants that provide a nesting place for a limited number of ant species, whilst the ants protect the myrmecophyte from defoliating arthropods and browsing mammals by stinging them. Natural selection has allowed ants that are known to have a painful sting to survive in such a habitat to the extent that some ants from the genera *Pseudomyrmex* and *Tetraponera* are obligate inhabitants of myrmecophytes. In some cases, ants use their venom in unusual ways. For example, *Pachycondyla tridentata* ants produce a foaming venom when disturbed and use their venom to paralyse their prey. This release of foam is a defence mechanism which is very effective against other small ants (Maschwitz et al., 1981).

It is therefore clear that ants have evolved venoms containing numerous toxins to induce pain, discomfort, paralysis and/or death in vertebrate and arthropod predators or prey. This is because protection of the nest, particularly protection of the brood and the queen, is a major concern for worker ants.

2.3. Antimicrobial properties of ant venoms

Ants are eusocial insects that typically live in colonies of relatives with a high population density. This increases the risk of introduction and spread of microbial pathogens. Consequently, ants have evolved strategies to inhibit microbial infections including the development and use of antimicrobial peptides. Firstly, predatory ant species may use their venom to inhibit internal pathogens present in captured prey that are brought back to the colony. In this way, the venom may protect the colony from infections following consumption of the prey species. In the ant venoms studied so far, this activity has been attributed to abundant linear, polycationic cytolytic peptides (see Section 3.1) that demonstrate potent antibacterial activity against both Gram-positive and Gram-negative bacteria (Cologna et al., 2013; Davies et al., 2004; Inagaki et al., 2004; Johnson et al., 2010; Kuhn-Nentwig, 2003; Mackintosh et al., 1995, 1998; Orivel et al., 2001; Rifflet et al., 2012; Viljakainen and Pamilo, 2008; von Sicard et al., 1989; Zelezetsky et al., 2005). More recently, similarity searches of ant genomes have revealed a number of tachystatins (antimicrobial chitin-binding peptides) with an inhibitor cystine knot (ICK) fold, as well as proline-rich abaecin-like, glycine-rich hymenoptaecin-like, insect defensin-like, and crustin-like antimicrobial peptides (Zhang and Zhu, 2012). These peptides may be part of the uncharacterized antimicrobial secretions from the thoracic metapleural or other glands that are spread over certain ants and the nest (Mackintosh et al., 1999; Yek and Mueller, 2011). Nevertheless, there is no evidence that these peptides are present in ant venoms.

3. Ant venom peptides

Alkaloid-rich ant venoms have been well-studied, particularly among the genera *Solenopsis* (Brand, 1978; Jones et al., 1996) and *Monomorium* (Jones et al., 1982, 2003, 2009, 1988). However, proteinaceous venoms remain highly understudied despite the fact that they appear to be very common in both the Poneroid and Formicoid clades of ant venoms. Thus, venoms from Poneroid ants have been shown to be rich in peptides especially venoms from the subfamilies Ponerinae (Cologna et al., 2013; Johnson et al., 2010; Orivel et al., 2001; Torres et al., 2014; Touchard et al., 2014a) and Paraponerinae (Piek et al., 1991a, 1991b; Rykaczewska-Czerwinska et al., 2008). Peptides have also been characterized from the venoms of Formicoid ants belonging to the subfamilies Myrmicinae (Bouzid et al., 2013; Rifflet et al., 2012), Myrmecinae (Davies et al., 2004; Inagaki et al., 2004, 2008a; Lewis et al., 1968; Mackintosh et al., 1998; Wiese et al., 2006; Wu et al., 1998), Pseudomyrmecinae (Touchard et al., 2014b) and Ectatomminae (Arseniev et al., 1994; Nolde et al., 1995; Pluzhnikov et al., 1999).

Peptides are the dominant compounds in most animal venoms and they represent a huge source of structurally diverse and

biologically active toxins with high potency and selectivity for a range of targets (King and Hardy, 2013). Despite the clear potential that ant venom peptides represent, their investigation and characterisation remains highly underexplored. To date, only 72 ant venom peptides, from 11 ant species, have been fully sequenced (Fig. 3). This is a very small number in comparison to snakes, cone snails, scorpions or spiders. For example, 922 spider peptide toxins have currently been sequenced from 86 spider species and are available in the ArachnoServer 2.0 (Herzig et al., 2011). Therefore, it has been estimated that more than 98% of arachnid venoms remain completely uncharacterized (Quintero-Hernández et al., 2011), and with ant venoms this figure would be closer to 99.9%.

Until recently, the main reason for the limited number of studies on ant venoms is the small size of ants, and hence the small yield of venom. However, advancements in analytical techniques, particularly in mass spectrometric technologies, has resulted in higher sensitivity and resolving power, allowing for a more extensive exploration of the ant venom peptidome. This review summarizes the current knowledge on the biochemical and pharmacological properties of all peptide toxins sequenced from ant venoms to date.

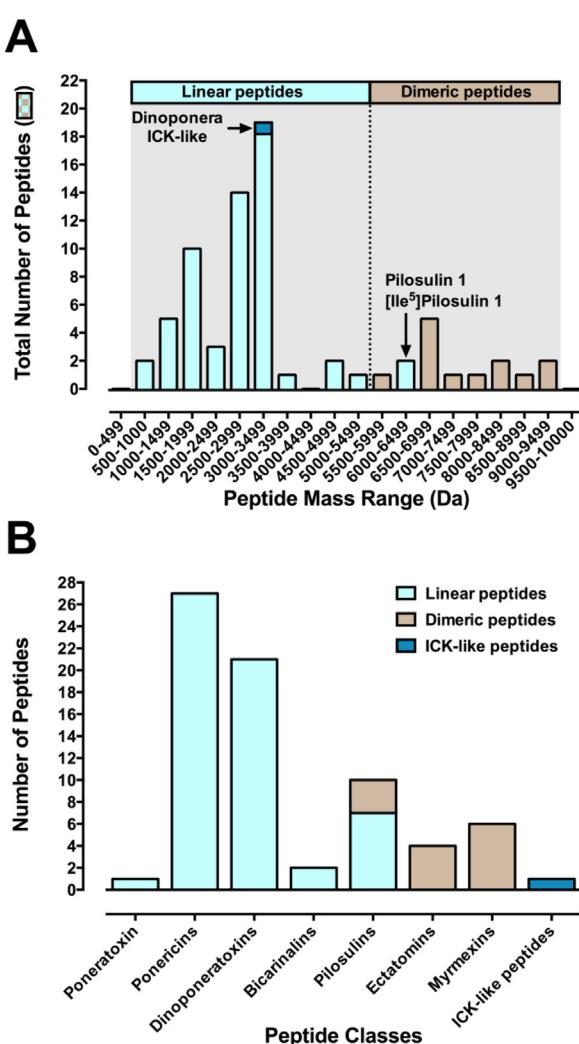


Fig. 3. (A) Bimodal mass distribution of the 72 characterized peptide toxins from ant venoms. Linear peptides range in mass from 761 to 5275 Da (except pilosulin 1, 6048 Da and [Ile^5]pilosulin 1, 6062 Da), while dimeric peptides range from 5603 to 9419 Da. (B) Ant peptide toxin classes. In both panels: cyan, linear peptides; beige, dimeric peptides; teal, ICK-like peptides. For clarification of colours in this figure, refer to the web version of this article.

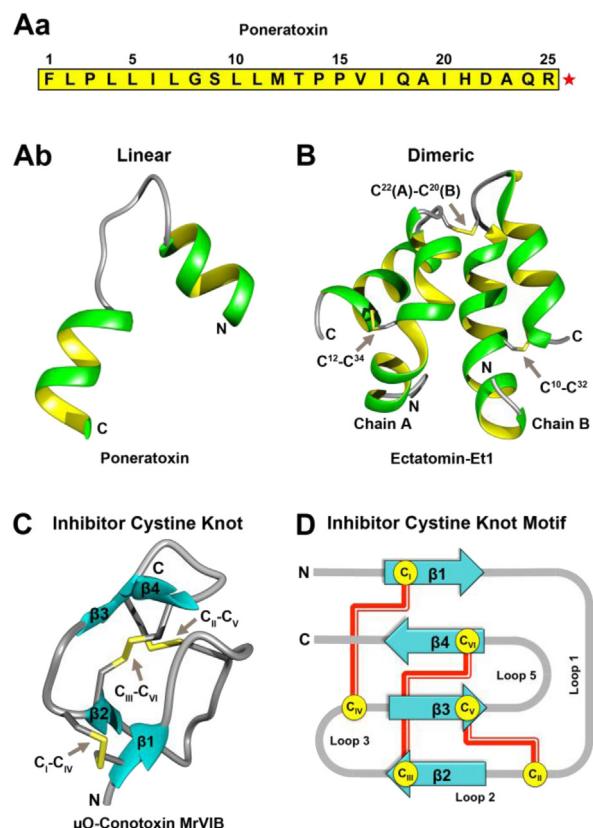


Fig. 4. Structures of ant peptide toxins. (Aa) Poneratoxin is a 2754.60 Da linear peptide (UniProtKB Accession POTX_PARCV) with no sequence homology to other peptides. The red star represents C-terminal amidation. (Ab) NMR structure of poneroxin (PDB Accession 1G92) shows it comprises of two α -helices. (B) NMR structure of ectatommin (PDB Accession 1ECI), a heterodimeric peptide that forms a four- α -helical bundle structure. The intra- and interchain disulfide bonds are labelled for clarity. (C) Homology model of *Dinoponera* ICK-like peptide modelled on μ O-conotoxin MrVIB from the venom of the cone snail *Conus marmoreus* (PDB Accession 1RMK; UniProtKB Accession CO16B_CONMR). In all panels, the peptide backbone is shown as a grey tube; β -sheets are represented by cyan arrows, α -helices are depicted as green/yellow spirals and disulfide bonds are shown as yellow tubes. The N-terminus (N) and C-terminus (C) of each peptide are also labelled. (D) Schematic representation of an ICK-like peptide. The pseudo-knot is formed when one disulfide bridge ($\text{C}_{\text{II}}-\text{C}_{\text{VI}}$) passes through a ring formed by two other disulfides ($\text{C}_{\text{I}}-\text{C}_{\text{IV}}$ and $\text{C}_{\text{II}}-\text{C}_{\text{V}}$) and the intervening backbone. For clarification of colours in this figure, refer to the web version of this article.

For the purposes of this review, these peptides have been classified based on their structure and classified into three main groups; (i) linear, (ii) dimeric and (iii) inhibitor cystine knot (ICK)-like peptides.

3.1. Linear peptides

Most of the proteomic studies on ant venoms have so far revealed that the majority of the proteinaceous component of ant venoms are small, polycationic linear peptides with masses below 5 kDa (Cologna et al., 2013; Johnson et al., 2010; Orivel et al., 2001; Rifflet et al., 2012). This is consistent with studies performed on other hymenopteran (wasp and bee) venoms (Argiolas and Pisano, 1985; Baptista-Saideberg et al., 2011; de Souza et al., 2004; Dias et al., 2014; Favreau et al., 2006; Gomes et al., 2014; Mendes et al., 2004; Qiu et al., 2012). Many of these linear peptides have antimicrobial properties and some possess additional insecticidal activity. Examples include ponerins from the neotropical ant *Pachycondyla goeldii* (Orivel et al., 2001) now reclassified as *Neoponera goeldii* (Schmidt and Shattuck, 2014), certain dinoponeratoxins (from *Dinoponera australis*) (Cologna et al., 2013) and pilosulins from the Australian jack jumper ant *Myrmecia pilosula*, which have been shown to have antimicrobial activity (Inagaki et al., 2004; Zelezetsky et al., 2005). These antimicrobial peptides demonstrate broad spectrum antibacterial activity and include α -helix antimicrobial peptides, and peptides with homology to the antimicrobial mucroporins, cecropins, brevinins, gaegurins, temporins and demaseptins (Cologna et al., 2013; Davies et al., 2004; Inagaki et al., 2004; Johnson et al., 2010; Kuhn-Nentwig, 2003;

Mackintosh et al., 1995, 1998; Orivel et al., 2001; Viljakainen and Pamilo, 2008; von Sicard et al., 1989; Zelezetsky et al., 2005).

3.1.1. Poneratoxin

In 1991, the first ant venom peptide toxin, ponera toxin, was isolated and sequenced (Piek et al., 1991b). Ponera toxin is a 25-residue peptide neurotoxin derived from the bullet ant *Paraponera clavata* (subfamily Paraponerinae) with no apparent homology to other known peptides (Fig. 4Aa) (Piek et al., 1991a). The 3D NMR structure of ponera toxin has also been determined (Szolajka et al., 2004) and revealed a 'V'-shaped peptide with two α -helices connected by a β -turn (Fig. 4Ab). It has been shown to modulate voltage-gated sodium (Nav) channels of both vertebrates and invertebrates and blocks synaptic transmission in the insect CNS. Ponera toxin induces long-lasting plateau action potentials and repetitive firing due to the presence of a slowly developing inward sodium current that activates at hyperpolarising potentials. This results from a potential toxin-induced interconversion between a fast and a slow conducting state of the Nav channel (Duval et al., 1992; Hendrich et al., 2001; Szolajka et al., 2004).

3.1.2. Ponerins

Ponerins are a group of 27 peptides characterised from the venom of the ponerine ants, *Pachycondyla goeldii* (Orivel et al., 2001), *Pachycondyla apicalis*, *Pachycondyla inversa* (Orivel, 2000) and *Pachycondyla commutata* (Touchard and Aili, unpublished data) (now all renamed *Neoponera* spp.; Schmidt and Shattuck, 2014). Ponerins possess amphipathic α -helical structures in polar environments, and have been shown to exhibit haemolysis,

Ponerin W Family	1	5	10	15	20	25
Ponerin W1	W	L	G	S	A	L
Ponerin W2	W	L	G	S	A	K
Ponerin Pa II 2	W	L	G	S	A	K
Ponerin Pa II 1	F	L	G	A	L	K
Ponerin W5	F	L	G	M	K	K
Ponerin-W-like 32.1	I	F	G	S	L	Q
Ponerin-W-like 32.2	F	I	G	S	L	Q
Melittin-like peptide	A	I	F	G	S	L
Brevinin-1HSa	F	I	G	S	A	K
Ponerin W4	F	L	G	A	V	R
Ponerin PI II2	G	I	W	G	T	A
Ponerin W3	F	G	H	L	K	G
Gaegurin-5	F	G	H	L	K	A
Venom antimicrobial peptide-9	F	G	H	L	K	T
Ponerin W6	F	G	H	L	K	A
Melittin-like peptide	F	G	H	L	K	A
Ponerin PI II1	F	G	H	L	K	A
Melittin	G	I	W	G	T	A
Melittin	G	I	W	G	T	A
Brevinin-1	F	L	G	A	V	P
Brevinin-1PTb	F	L	G	A	V	P

Ponerin L Family	1	5	10	15	20	24
Ponerin L2	L	L	K	E	L	*
Ponerin L1	L	L	K	E	L	*
Ponerin PI III1	L	L	K	E	L	*
Dermaseptin-J6	L	L	K	E	L	*
Dermaseptin-J5	G	L	K	E	L	*
Dermaseptin-DRG2	R	G	L	E	L	*
Dermaseptin-5	G	L	K	E	L	*
P17 (Bicarinalin 2)	L	F	K	E	L	*
Ponerin Pa IV1	G	K	D	V	L	R
Bicarinalin 1	K	I	P	W	G	K

Ponerin G Family	1	5	10	15	20	25	30
Ponerin G1	G	W	K	D	W	A	K
Ponerin PI 13	G	W	R	D	W	K	A
Ponerin G3	G	W	R	D	W	K	A
Ponerin G5	G	W	K	D	W	K	A
Ponerin PI 2	G	W	K	D	W	K	A
Ponerin G2	G	W	K	D	W	K	A
Ponerin PI 11	G	W	K	D	W	K	A
Ponerin PI 14	G	W	K	D	W	K	A
Ponerin G4	D	F	K	D	W	K	A
Ponerin Pa I1	G	F	K	D	W	K	A
Ponerin Pa I2	G	F	D	M	L	R	A
Cecropin B	K	W	I	F	K	E	V
Cecropin B	R	W	I	F	K	E	V
Cecropin-A	R	W	I	F	K	E	V
Cecropin	R	W	I	F	K	E	V
Ponerin G7	G	L	V	D	L	G	A
Ponerin G6	G	L	V	D	L	G	A

% I	% S	UniProtKB ID	Organism	M _{calc} (Da)
100	100	PCW1_PACGO	<i>Pachycondyla goeldii</i>	2708.68
92	92	PCW2_PACGO	<i>Pachycondyla goeldii</i>	2708.68
84	88	Orivel_2000	<i>Pachycondyla apicalis</i>	2695.69
72	84	Orivel_2000	<i>Pachycondyla apicalis</i>	2585.59
60	76	PCW5_PACGO	<i>Pachycondyla goeldii</i>	2588.58
56	76	PCWL1_LYCMC	<i>Lychas mucronatus</i>	2826.65
50	73	PCWL2_LYCMC	<i>Lychas mucronatus</i>	2911.71
44	68	MLP_RANTE	<i>Rana temporaria</i>	2310.38
32	68	BRI1A_ODHO	<i>Odorranas hisoi</i>	2571.48
54	65	PCW4_PACGO	<i>Pachycondyla goeldii</i>	2862.70
46	65	Orivel_2000	<i>Pachycondyla inversa</i>	2824.75
46	65	PCW3_PACGO	<i>Pachycondyla goeldii</i>	2862.77
40	64	GGN5_GLARU	<i>Glandirana rugosa</i>	2548.46
28	64	NDB59_MESEU	<i>Mesobuthus eupeus</i>	2390.40
40	60	PCW6_PACGO	<i>Pachycondyla goeldii</i>	2029.23
36	60	MLP_RANAR	<i>Rana arvalis</i>	2361.43
32	60	Orivel_2000	<i>Pachycondyla inversa</i>	2042.27
31	58	MEL_APFL	<i>Apis florea</i>	2816.75
31	58	MEL_APDO	<i>Apis dorsata</i>	2845.74
28	56	BRI1_BRNBP	<i>Rana brevipoda porsa</i>	2527.48
28	52	BRI1P_RANPC	<i>Rana picturata</i>	2451.32

% I	% S	UniProtKB ID	Organism	M _{calc} (Da)
100	100	PCL2_PACGO	<i>Pachycondyla goeldii</i>	2575.57
96	100	PCL1_PACGO	<i>Pachycondyla goeldii</i>	2593.62
96	96	Orivel_2000	<i>Pachycondyla inversa</i>	2603.70
39	48	DMS6_PHAJA	<i>Phasmahyla jandaia</i>	2765.59
39	48	DMS5_PHAJA	<i>Phasmahyla jandaia</i>	2764.61
39	48	DRG2_PHYBI	<i>Phyllomedusa bicolor</i>	2908.66
35	45	DMS5_PHYSA	<i>Phyllomedusa sauvagei</i>	2838.68
25	38	Rifflet et al., 2012	<i>Tetramorium bicarinatum</i>	1571.02
12	26	Orivel_2000	<i>Tetramorium bicarinatum</i>	3909.08
12	24	Rifflet et al., 2012	<i>Tetramorium bicarinatum</i>	2212.33

% I	% S	UniProtKB ID	Organism	M _{calc} (Da)
100	100	PCG1_PACGO	<i>Pachycondyla goeldii</i>	3211.76
83	90	Orivel_2000	<i>Pachycondyla inversa</i>	3204.84
73	80	PCG3_PACGO	<i>Pachycondyla goeldii</i>	3380.89
73	80	PCG5_PACGO	<i>Pachycondyla goeldii</i>	3106.78
67	80	Orivel_2000	<i>Pachycondyla inversa</i>	3331.94
70	77	PCG2_PACGO	<i>Pachycondyla goeldii</i>	3305.88
70	77	Orivel_2000	<i>Pachycondyla inversa</i>	3337.82
70	77	Orivel_2000	<i>Pachycondyla inversa</i>	3193.81
63	73	PCG4_PACGO	<i>Pachycondyla goeldii</i>	3162.67
60	73	Orivel_2000	<i>Pachycondyla apicalis</i>	3046.66
60	70	Orivel_2000	<i>Pachycondyla apicalis</i>	2910.69
37	51	CECB_ANPTE	<i>Antheraea pernyi</i>	3814.33
34	49	ATUDN2_BOMMO	<i>Bombyx mori</i>	3892.27
31	49	CECA_HELVI	<i>Heliothis virescens</i>	3900.34
31	49	CEC4_BOMMO	<i>Bombyx mori</i>	3745.20
30	33	PCG7_PACGO	<i>Pachycondyla goeldii</i>	1875.19
27	30	PCG6_PACGO	<i>Pachycondyla goeldii</i>	1817.19

Fig. 5. Sequence alignment of bicarinalin and three ponerin families of linear peptides. Toxin names boxed in light grey are derived from ants. Identical residues in the peptide sequences are boxed in yellow while conservative substitutions are shown in red italic text. Cysteines are highlighted in black while red stars represent C-terminal amidation. Gaps were introduced to optimize the alignments. Percentage identity (%I) is relative to the first peptide of each family, while percentage similarity (%S) includes conservatively substituted residues. M_{calc}, Theoretical monoisotopic mass calculated using GPMAW 9.20 software. Note added in proof: *Pachycondyla* spp. listed in this figure have all recently been renamed *Neoponera* spp. (Schmidt and Shattuck, 2014). For clarification of colours in this figure, refer to the web version of this article.

antibacterial activity against both Gram-positive and Gram-negative bacteria, as well as insecticidal activity (Orivel et al., 2001). Ponerins have been classified into three different families ('G', 'W' and 'L') based on sequence homology (Fig. 5). The ponerins show considerable sequence homology with other previously characterised peptides. For example, ponerin G peptides show homology to cecropin-like peptides from moths, flies, beetles and butterflies (Lee et al., 2013); ponerin W peptides have homology with the cytolytic peptide bee peptide melittin and gaegurins from frogs (Lee et al., 2011; Palma, 2013); and ponerin L peptides with dermaseptins isolated from the skin of *Phasmahyla* and *Phyllomedusa* frogs (Amiche and Galanth, 2011; Nicolas and Amiche, 2013). Given the known actions of these other peptides as cytolytic agents, ponerins may also form amphipathic α -helical structures in cell membranes, although only ponerin W peptides appear to have additional haemolytic actions. This function may be important in preventing the spread of microbial pathogens in ant colonies following ingestion of contaminated prey or their introduction into their colony following paralysis and subsequent transport of the prey into the colony (Lai et al., 2012).

3.1.3. Dinoponeratoxins

The giant Neotropical hunting ant *Dinoponera australis* (sub-family Ponerinae) is a solitary foraging, predatory ant whose venom paralyses invertebrates and causes a range of systemic effects in vertebrates (Haddad Junior et al., 2005). Envenomation in humans is rare, although stings have been reported to produce rapid and excruciating pain, diaphoresis, nausea, vomiting, tachycardia and lymphadenopathy (Haddad Junior et al., 2005). Liquid chromatography–mass spectrometry (LC-MS) analysis of the venom identified over 75 proteinaceous components with numerous small mass peptides (429–3214 Da) and a wide range of hydrophobicity and abundance. The six most abundant peptides were sequenced by tandem MS and Edman degradation and named dinoponeratoxins ('Da' toxins) (Johnson et al., 2010). Subsequently similar dinoponeratoxin peptides have been isolated and sequenced from the related ant *Dinoponera quadriceps* – henceforth known as 'Dq' toxins (Cologna et al., 2013). All 21 Dq and Da dinoponeratoxins show various degrees of homology with existing linear peptides, and can be separated into six groups (Fig. 6).

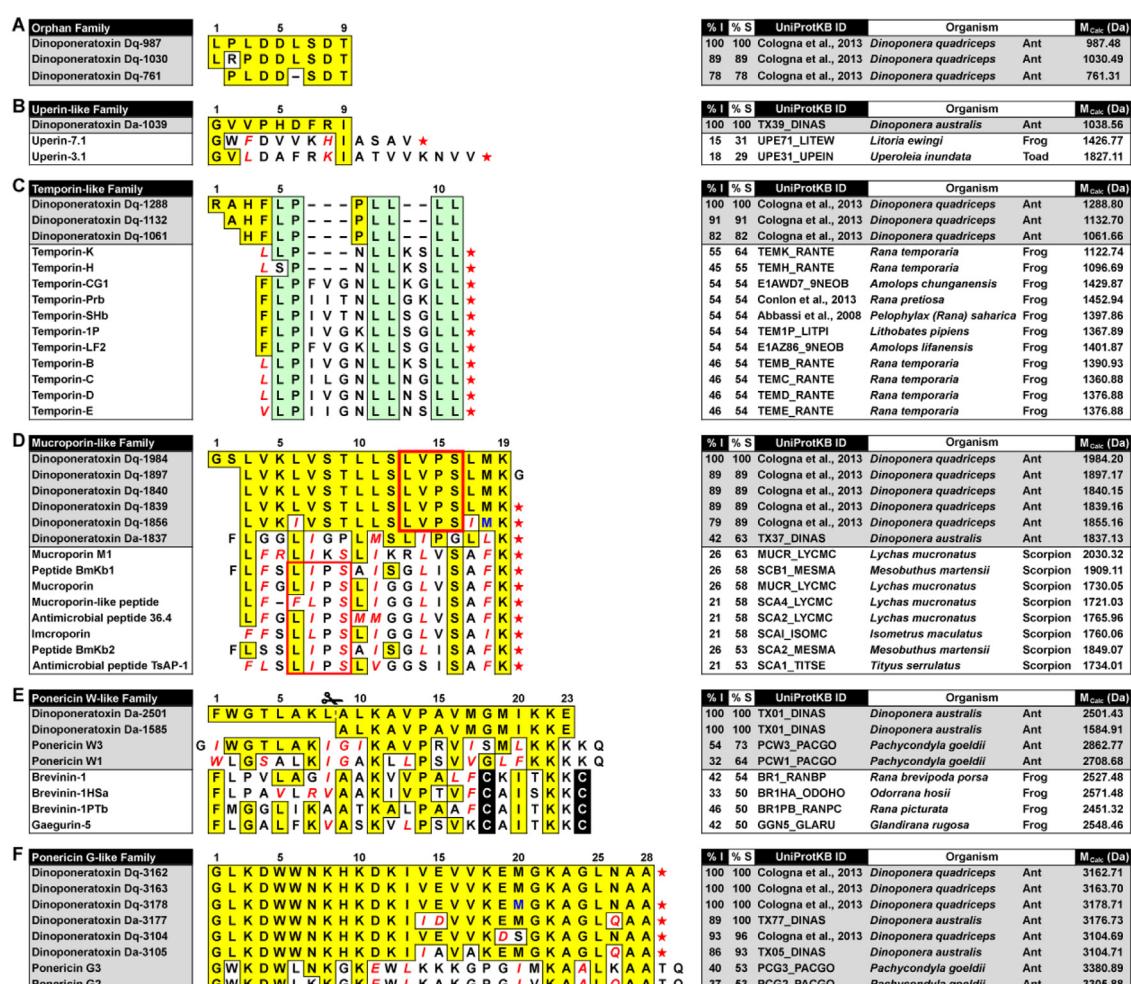


Fig. 6. Alignment of the dinoponeratoxin families of linear peptides. Toxin names boxed in light grey are derived from ants. Identical residues are boxed in yellow while conservative substitutions are shown in red italic text. Numbering is according to the first peptide in each family. Gaps were introduced to optimize the alignments. Red stars indicate amidated C-terminus and cysteines are highlighted in black. Percentage identity (%) is relative to the first peptide in each alignment while percentage similarity (%) includes conservatively substituted residues. Apart from uperin peptides in panel B, only homologies greater than 50% are displayed. M_{Cal}. Theoretical monoisotopic mass calculated using GPMWA 9.20 software. (C) Residues Leu⁵, Pro⁶, and Leu^{8–11} (numbering from Dq-1288), a common motif within temporin peptide families, are highlighted in green. (D) The residues K(V/L/I)IPS within the red boxes are thought to be critical for function in the scorpion antimicrobial peptide pandinin 2 and the scorpion peptides in panel D (Harrison et al., 2014). (E) Dinoponeratoxin Da-2501 is cleaved at the position marked to generate Da-1585. The blue Met¹⁸ in Dinoponeratoxin Dq-1837 (panel D) and Met²⁰ in Dinoponeratoxin Dq-3178 (panel F) indicate residues that are probably oxidized. Note: *Pachycondyla goeldii* has recently been renamed *Neoponera goeldii* (Schmidt and Shattuck, 2014). For clarification of colours in this figure legend, refer to the web version of this article.

Group I are short 7–9 residue Dq peptides forming a three-member orphan peptide family with no homology to existing peptides (Fig. 6A), and no known biological activity (Cologna et al., 2013). Group II has only one member, Da-1039 (Fig. 6B), with only very limited homology to the uperin family of antibacterial frog skin secretions (Bradford et al., 1996; Steinborner et al., 1997). Group III comprise three 9–11 residue Dq toxins with moderate homology with the temporin family of antibacterial frog skin secretions (Abbassi et al., 2008; Rinaldi and Conlon, 2013; Simmaco et al., 1996). Temporins are one of the largest groups of antimicrobial peptides within the cationic host defence peptide family. They were originally isolated from skin secretions of the frog *Rana temporaria*, and are amphipathic α -helical peptides of 8–19 residues with a low net positive charge (0 to +3) and C-terminal amidation (Mangoni et al., 2007; Suzuki et al., 2007). The reasonably high homology of the temporin-like Dq toxins would suggest potentially similar biological activity, especially given the conservation of the common Pro and Leu residues found in temporin peptide families (Simmaco et al., 1996; residues in green boxes in Fig. 6C). This leucine-rich tail has previously been shown to be important for membrane interaction (Avitabile et al., 2013). The antimicrobial activity of temporins is associated with an alteration of the cytoplasmic membrane permeability, without destruction of cell integrity (Mangoni et al., 2004). Temporins are particularly active against Gram-positive bacteria but most do not affect eukaryotic cells. However, they may act in a more complex way to inhibit various metabolic functions of the cell (Epand and Vogel, 1999; Park et al., 1998).

Group IV is the largest group of dinoponeratoxins and have masses between 1837 and 1984 Da with 17–19 residues. These have significant homology (53–63% similarity) with the antibacterial cationic host defence peptides BmKb (caerin-like) and mucroporin originally isolated from the venom of the scorpions *Mesobuthus martensii* and *Lychas mucronatus*, respectively (Dai et al., 2008; Zeng et al., 2004). These antimicrobial peptides are now found in a range of scorpion species and are being investigated as novel anti-infective drugs or lead compounds, for treating antibiotic-resistant microbial infections (Harrison et al., 2014).

Group V is a recently discovered collection of 15 dinoponeratoxins from the venom of *Dinoponera quadriceps* sequenced from a total of 354 peptides found in this venom (Cologna et al., 2013). These were found to share homology with the ponerin W family, dinoponeratoxins (from *D. australis*) and poneratoxin. These

peptides also revealed both antimicrobial and antifungal activities (Cologna et al., 2013).

Group VI comprises of the ant venom peptides Da-3105 and Da-3177, from the giant Neotropical hunting ant *D. australis*, which show considerable homology to ponerin G2 and may possess similar bioactivity.

3.1.4. Bicarinalins

Two novel peptides, bicarinalin 1 and P17 (bicarinalin 2) have been isolated and characterised from the venom of the ant *Tetramorium bicarinatum* (Myrmicinae) from a total of 31 peptides identified in this venom (Rifflet et al., 2012). Interestingly, these peptides show very low homology with known peptide toxins (Fig. 5). Bicarinalin 1 exhibits all the characteristics of an amphipathic helical peptide and has broad and potent antibacterial activity similar to melittin, pilosulin and defensin but with weaker haemolytic activity (Rifflet et al., 2012; Téné et al., 2014). Accordingly, it is being investigated as an anti-infective agent for use against emerging antibiotic-resistant pathogens. Recently the venom gland transcriptome of *Tetramorium bicarinatum*, one of the world's most broadly distributed ant species, has also been published (Bouzid et al., 2013). Transcribed *T. bicarinatum* venom gland ESTs revealed allergenic/cytotoxic peptides, with homology to pilosulins 1, 3 and 5, and paralytic peptide toxins, one of which possesses homology with the insect cytokine precursor uENF2. These allergenic/cytotoxic and paralytic toxins contributed close to 70% of the total EST cDNAs.

3.2. Dimeric peptides

Dimeric peptides are peptides with two subunits that are linked covalently with a disulfide bond (Sarray et al., 2013) and peptide dimerization is currently being investigated as a potential way to increase the activity of certain peptide toxins (Vizzavona et al., 2009). Except for snake venoms (Osipov et al., 2008), a dimeric scaffold in peptides is quite rare in venomous animals, although it has occasionally been reported in the venoms of some scorpions (Zamudio et al., 1997), spiders (Santos et al., 1992) and marine cone snails (Loughnan et al., 2006). In the case of ant venoms, dimeric peptides seem to be common in the subfamilies Ectatomminae, Myrmecinae and Pseudomyrmecinae (see below), but have not yet been described in other subfamilies. The amino acid sequences and

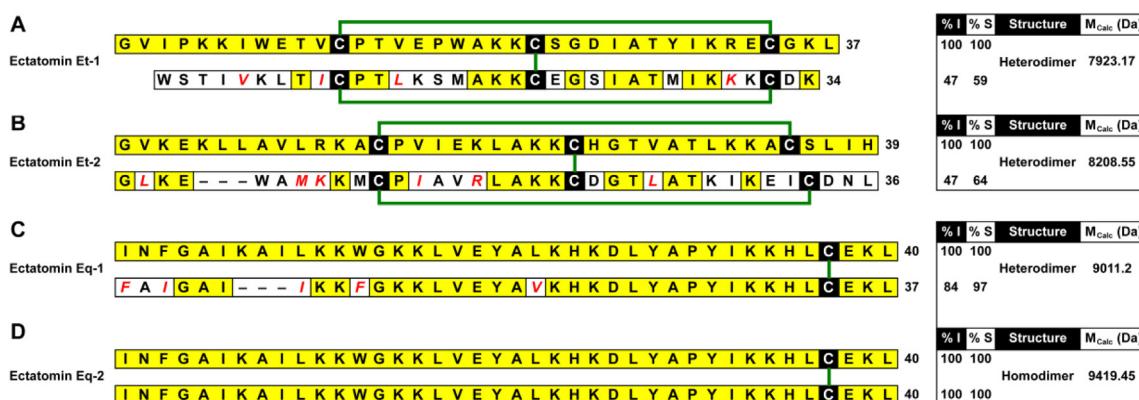


Fig. 7. Sequences and structures of the ectatommin family of dimeric ant peptides. Identical residues are boxed in yellow while conservative substitutions are shown in red italic text. Cysteines are highlighted in black and the predicted disulfide-bonding pattern is shown in green between the sequences. Gaps were introduced to optimize the alignments. Percentage identity (%I) is relative to the longer (upper) chain for each peptide while percentage similarity (%S) includes conservatively substituted residues. M_{Calc}, Theoretical monoisotopic mass calculated using GPMW 9.20 software. The heterodimeric ectatommin Et peptides (A–B) are from *Ectatomma tuberculatum* while ectatommin Eq peptides (C–D) are from *Ectatomma brunneum* (formerly *E. quadrident*). (A) Sequences for ectatommin Et-1 toxins are from UniProtKB Accessions ECAA_ECTTU and ECAB_ECTTU. (B–D) Remaining sequences are from Pluzhnikov et al. (2000). For clarification of colours in this figure, refer to the web version of this article.

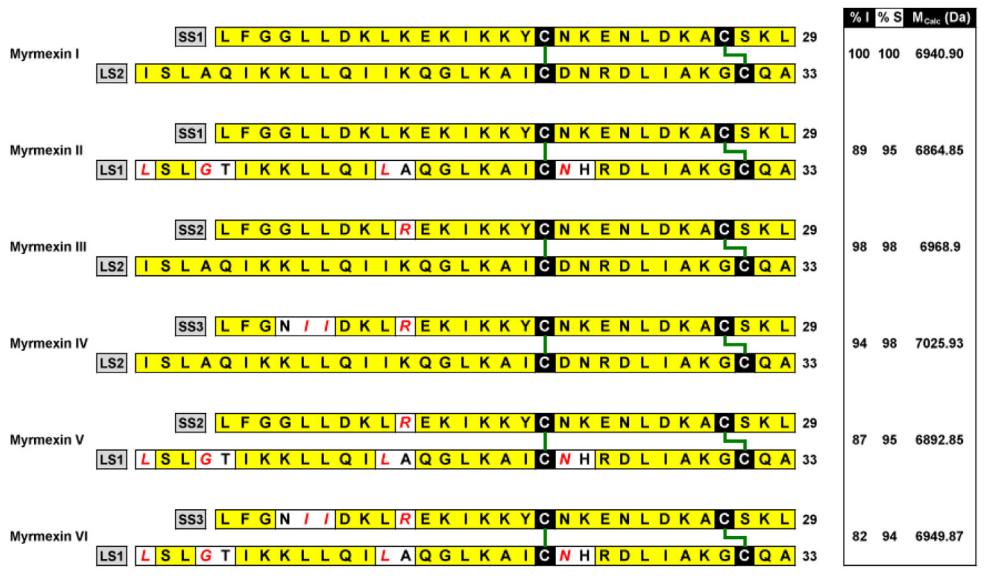


Fig. 8. Sequences and structures of the myrmexin family of heterodimeric peptides from the venom of the ant *Pseudomyrmex triplarinus*. Each myrmexin is composed of a short subunit (SS1, SS2 or SS3; grey boxes) and a long subunit (LS1 or LS2; grey boxes) linked by two disulfide bonds. Identical residues are boxed in yellow while conservative substitutions are shown in red italic text. Cysteines are highlighted in black and the predicted inter-chain disulfide-bonding pattern is shown in green between the sequences. Gaps were introduced to optimize the alignments. Percentage identity (%I) is relative to myrmexin I while percentage similarity (%S) includes conservatively substituted residues. M_{Calc}, Theoretical monoisotopic mass calculated using GPMW 9.20 software. Sequences are taken from Pan and Hink (2000). For clarification of colours in this figure, refer to the web version of this article.

disulfide connectivity of the known dimeric peptide-toxins are reported in Figs. 7–9.

3.2.1. Ectatomins

One of the most potent neurotoxic peptides isolated from ant venoms, is ectatomin (Et-1), from the venom of the ant *Ectatomma*

tuberculatum (Ectatomminae) (Pluzhnikov et al., 1994) and its homologue, ectatomin Et-2 (Pluzhnikov et al., 2000). These peptides are highly basic heterodimeric complexes consisting of two highly homologous amphiphilic polypeptide chains linked together by one inter-chain disulfide bond (Arseniev et al., 1994). Each chain also possesses an intra-chain disulfide bond (Fig. 7A–B). Disulfide

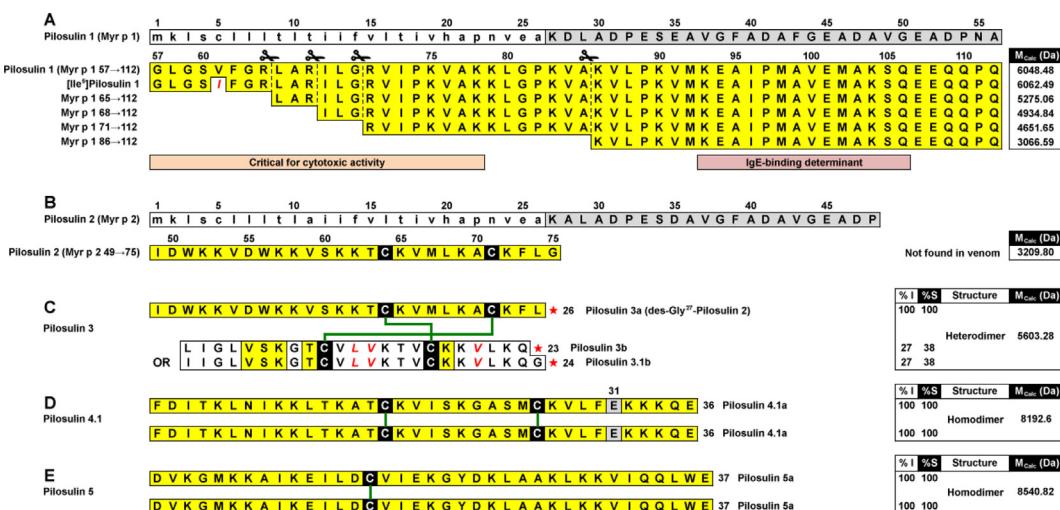


Fig. 9. Sequences and structures of the pilosulin family of linear and dimeric ant peptides. (A–B) Translated sequences of the linear peptides pilosulin 1 (A; from *Myrmecia pilosula*; UniProtKB accession MYR1_MYRPI) and pilosulin 2 (B; from *Myrmecia pilosula*; UniProtKB accession MYR3A_MYRPI). Sequences represent complete preproteptides, where signal peptides are boxed in white and in lowercase, propeptide sequences are boxed in gray and the mature peptides are boxed in yellow. (A) In addition to the natural variant [Ile⁵] pilosulin 1, pilosulin 1 undergoes cleavage at the sites marked above the mature peptide sequence to yield four additional peptides, while the residues important for cytotoxic activity and IgE binding are highlighted beneath the sequences. (B) Pilosulin 2 does not appear to be found in venom but undergoes post-translational modification to form the monomer pilosulin 3a (des-Gly²⁷-Pilosulin 2) that forms the heterodimer pilosulin 3 (C) with the monomer pilosulin 3b (MYR3B_MYRPI). A natural variant, pilosulin 3.1b, can be found in the venom of *Myrmecia banksi* (MYR3_MYRBA). (D–E) Pilosulin 4.1 (Wiese et al., 2006) and pilosulin 5 (MYR5_MYRBA) are homodimers from *Myrmecia banksi*. In the case of pilosulin 4.1, cDNA cloning predicted a homodimer of pilosulin 4a (MYR4_MYRBA), but this was not detected in venom and the [Glu³¹]pilosulin 4 variant (pilosulin 4.1a) found in venom is shown. For the dimeric peptides (C–E), cysteines are highlighted in black and the predicted disulfide-bonding pattern is shown in green between the sequences. Red stars indicate an amidated C-terminus. M_{Calc}, Theoretical monoisotopic mass calculated using GPMW 9.20 software. For clarification of colours in this figure, refer to the web version of this article.

bonds render venom peptides resistant to a number of different proteases and environmental extremes resulting in stable peptide toxins (King and Hardy, 2013). The three dimensional structure of Et-1 was determined by NMR and revealed that each ectatommin chain comprises two anti-parallel α -helices linked by a hinge region of four amino acid residues and a disulfide bridge (Fig. 4B) (Nolde et al., 1995). Two other ectatommins (Eq-1 and Eq-2) have also been isolated from the venom of *Ectatomma brunneum* (previously *Ectatomma quadridens*). These novel Eq ectatommins are also dimeric and linked by one inter-chain disulfide bond. However, they lack the intra-chain disulfide bond present in Et-1 and -2 (Pluzhnikov et al., 2000).

Et-1 appears to account for the major toxic effect of *Ectatomma tuberculatum* venom causing toxic effects in both mammals and insects (Pluzhnikov et al., 1999). At high concentrations (0.50–1 μ M), Et-1 is a pore-forming peptide that inserts into cellular and artificial membranes but is not internalized. It produces haemolytic and cytolytic effects on rabbit erythrocytes, *Xenopus laevis* oocytes, rat cardiomyocytes and both insect and vertebrate cell lines. In *X. laevis* oocyte membranes, this arises due to the formation of nonselective cationic channels by two Et-1 molecules and appears to involve binding to lipids rather than a specific receptor. The increase in cell permeability, with resultant ion leakage, results in cell death (Pluzhnikov et al., 1994, 1999). At much lower concentrations (1–10 nM), Et-1 is capable of inhibiting whole-cell L-type calcium currents in isolated rat ventricular myocytes. Importantly, it prevents β -adrenoceptor- or adenylate cyclase-mediated activation of calcium currents suggesting that Et-1 interacts directly or allosterically with agonist-bound β -adrenoceptors preventing activation of calcium channels further down the signal transduction cascade. The modulation of calcium channels and possibly β -adrenoceptors by Et-1 may underlie its potent toxicity by interfering with the process of muscle contraction, neurotransmitter release and neuromodulation (Pluzhnikov et al., 1999).

3.2.2. Myrmexins

In vitro and clinical studies have shown that *Pseudomyrmex triplarinus* (Pseudomyrmecinae) ant venom decreases pain and inflammation in patients with rheumatoid arthritis and reduces swelling in animal models of inflammation (Altman et al., 1984; Hink and Butz, 1985; Schultz and Arnold, 1984). Myrmexins are a family of six related polypeptides (myrmexins I–VI) that have been purified from the venom of *Pseudomyrmex triplarinus*. These peptides are heterodimeric complexes comprising a combination of a short subunit of 29 residues (SS1, SS2 or SS3) and a long subunit of 33 residues (LS1 or LS2) stabilized by two inter-chain disulfide bonds (Pan and Hink, 2000) (Fig. 8). Unfortunately, it is not known at present which of the myrmexin peptides are associated with the anti-inflammatory activity observed with whole venom. Three additional myrmexin-like polypeptides from the venom of the related ant *Pseudomyrmex penetrator* (one heterodimeric and two homodimeric) have also been identified, however, they are yet to be sequenced (Touchard et al., 2014b). These myrmexins may represent a new class of toxins present in Pseudomyrmecine ants.

3.2.3. Pilosulins

Australian ants of the *Myrmecia pilosula* species complex (Myrmeciinae), also known as jack jumper ants, have a painful sting that is responsible for around 90% of life-threatening ant sting allergies in Australia (Brown et al., 2003; Douglas et al., 1998; Street et al., 1994). In South Eastern Australia around 2.7% of the population are allergic to *Myrmecia pilosula* venom, with approximately 50% of allergic people experiencing life-threatening reactions (Brown et al., 2003). The toxicity of the venom appears to result

from the presence of a variety of histamine-like, haemolytic and eicosanoid-releasing factors, peptides such as pilosulins, and enzymes including phospholipases, hyaluronidase, and phosphatases (Matuszek et al., 1994a, 1992, 1994b; McGain and Winkel, 2002).

Using cDNA sequencing, two major protein allergens from *Myrmecia pilosula* sharing a common leader sequence have been identified (Donovan et al., 1993, 1995, 1994; Street et al., 1996). They encode the 112 and 75 amino acid prepropeptides Myr p 1 and Myr p 2, respectively (Fig. 9A–B). Pilosulin 1, the mature peptide product from residue 57 to 112 of Myr p 1 (Myr p 1 57 → 112), is a 6048 Da linear allergenic basic peptide that exhibits haemolytic and cytotoxic activity and is one of the major allergens that have been identified in this venom (Donovan et al., 1993, 1994; Wu et al., 1998). However, pilosulin 1 exists mainly, and sometimes exclusively, as a Val5Ile substituted isoform known as [Ile⁵]pilosulin 1 (Davies et al., 2004) (Fig. 9A). Pilosulin 1 is also cleaved to form four additional N-terminally truncated isoforms with varying degrees of cytotoxic activity (Fig. 9A).

Pilosulin 2 (Myr p 2 49 → 75) has never been detected in whole venom in its monomeric form (Donovan and Baldo, 1997). However, a des-Gly²⁷ pilosulin 2 peptide (renamed pilosulin 3a) has been found as part of the 5603 Da heterodimeric peptide pilosulin 3. The additional subunit of pilosulin 3 from *Myrmecia pilosula* is the 23 residue pilosulin 3b (Davies et al., 2004), or the variant pilosulin 3.1b from *Myrmecia banksii* (Inagaki et al., 2004), thought to be part of the *Myrmecia pilosula* species complex (Imai et al., 1994) (Fig. 9C). Pilosulin 3 displays antimicrobial activity, and is the major allergen in *M. pilosula* venom, along with [Ile⁵]pilosulin 1 accounting for 80% of the total venom peptide content. Pilosulin 4a peptide was originally identified via cDNA cloning (Inagaki et al., 2004) but was not detected in venom, while its Asp31Glu variant pilosulin 4.1a was found to be present only as a homodimeric peptide, pilosulin 4.1 (Wiese et al., 2006) (Fig. 9D). cDNA cloning also revealed the presence of a novel bioactive dimeric peptide pilosulin 5 connected by a single disulfide bond. Synthetic pilosulin 5 dimer causes significant histamine release that may be related to the weak homology of the peptide to the wasp peptide mastoparan (Inagaki et al., 2008a).

Although the monomeric pilosulin peptides (pilosulin 2, 3.2b, 4 and 5) all show antibacterial and histamine-releasing activities (Inagaki et al., 2004, 2008a) and some pilosulins, particularly 3a and to a lesser extent 4.1 and [Ile⁵]pilosulin 1, are known to be highly allergenic (Wiese et al., 2007), the biological activities of these peptides have not been fully investigated.

3.3. ICK-like peptides

The inhibitor cystine knot (ICK) structural motif is an evolutionary conserved structure that has been found in plants, fungi, viruses, antimicrobial peptides from horseshoe crabs (tachystatins) and the venoms of many organisms such as spiders, scorpions, cone snails, insects (bees) and sea anemones (Barbault et al., 2003; Bloch and Cohen, 2014; Cammue et al., 1992; Gilly et al., 2011; Osaki et al., 1999; Pallaghy et al., 1994; Rodríguez et al., 2014; Zhu et al., 2003). The ICK motif is defined as an embedded ring formed by two disulfide bonds Cys(I–IV) and Cys(II–V) and their connecting backbone segments through which is threaded a third disulfide bond Cys(III–VI), forming a cystine knot. It is invariably associated with a nearby anti-parallel β -sheet and appears to be a highly effective motif for stabilizing peptide structures (Fig. 4D). Peptides with an ICK motif represent attractive scaffolds in drug design because of their inherent chemical stability and resistance to proteases provided by the fold and the wide range of amino acid sequences that can be accommodated in the structure (Craik et al., 2001; Norton and Pallaghy, 1998; Pallaghy et al., 1994; Zhu et al., 2003). While

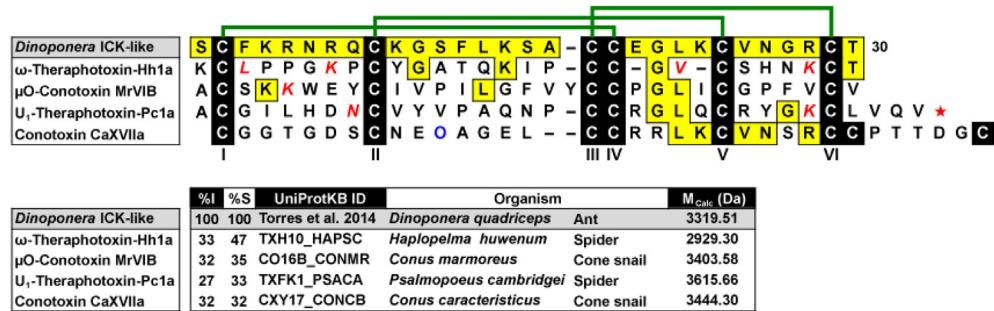


Fig. 10. Structure and sequence alignment of the *Dinoponera* ICK-like peptide. The upper panel shows the disulfide bonding connectivity and alignment with homologous peptides. Cysteines are highlighted in black and the predicted disulfide-bonding pattern, similar to other ICK peptides, is shown in green above the sequences. Identical residues are boxed in yellow while conservative substitutions are shown in red italic text. Gaps were introduced to optimize the alignments. Red stars indicate an amidated C-terminus while the blue O¹¹ in conotoxin CaVIIa indicates a hydroxyproline residue. The lower panel shows the percentage identity (%I) relative to *Dinoponera* ICK-like peptide while percentage similarity (%S) includes conservatively substituted residues. M_{calc}, Theoretical monoisotopic mass calculated using GPMAW 9.20 software. For clarification of colours in this figure, refer to the web version of this article.

large numbers of ICK peptide toxins have been reported from other arthropod venoms such as spiders and scorpions, only two types of ant venom peptides displaying this structural motif are currently known – *Dinoponera* ICK-like peptide and SKTXs.

3.3.1. *Dinoponera* ICK-like toxin

The recent transcriptome analysis of the venom glands of the ant *Dinoponera quadriceps* (Ponerinae) has confirmed the presence and sequence of the first ICK-like peptide in ant venoms (Torres et al., 2014). This *Dinoponera* ICK-like peptide is a minor component of the venom of *Dinoponera quadriceps* but has a VI/VII cysteine framework (–C–C–CC–C–C–) consistent with other ICK toxins (Fig. 4C). This peptide shows limited homology to the ICK toxins ω -theraphotoxin-Hh1a and μ O-conotoxin MrVIB peptides found in tarantula and cone snail venoms, respectively, both of which exhibit neurotoxic activity via activity on voltage-gated ion channels (Liu et al., 2006; McIntosh et al., 1995) (Fig. 10).

3.3.2. SKTXs

The venom of the ant *Strumigenys kumadori* (Myrmicinae) also possesses ICK-like peptides which have been named SKTXs (Inagaki et al., 2008b). SKTXs are thought to modulate Nav channels of *Drosophila*, however, this study remains unpublished and sequences of these peptides are still unknown.

4. Conclusion and perspectives

Until recently, the low yield of venom from ant species has severely restricted the biochemical and pharmacological characterisation of ant venom peptides. However, advances in the development of miniaturized bioassays and improvements in the sensitivity of mass spectrometry and NMR spectroscopy now allow broader investigations of the small quantities of venom peptides provided by small animals, especially ants. Indeed, mass spectrometry has been used as a method to improve the accuracy of taxonomic findings to reveal cryptic ant species within species complexes (Touchard et al., 2014a). This chemotaxonomic tool can therefore contribute to more rapid species identification and more accurate taxonomies.

The limited number of studies to date has revealed a number of unique structures across a broad range of ant subfamilies that differ from those described in other animal venoms. Given the diversity in ant species and distribution, ant venoms therefore represent vast sources of potentially novel bioactive toxins that could be exploited in drug and bioinsecticide discovery programs. For example, there

is increasing awareness that peptides represent an under-utilized source of lead compounds for new therapeutics. Arguably, the largest source of chemical diversity comes from peptides derived from animal venoms. In animal venoms the evolutionary pressure for improved prey capture and/or defence has resulted in complex preoptimised combinatorial peptide libraries with extremely diverse pharmacologies that interact with a wide range of molecular targets. The discovery that these peptides bind to their cognate receptors and ion channels with high affinity and selectivity means that many are now being investigated as sources of lead compounds in therapeutic discovery pipelines (Bosmans et al., 2009; Escoubas and King, 2009; Lewis and Garcia, 2003; Vetter et al., 2011; Vetter and Lewis, 2012). Hence, there is a growing number of novel peptide or peptidomimetic therapeutics appearing on the drug market, or in clinical trials, which are derived from toxins from the venoms of cone snails, snakes, Gila monster, scorpions, spiders and sea anemones. Ants could also provide a unique source of potential therapeutic leads, especially antimicrobials and neuro-active compounds.

Since some venomous animals, particularly arachnids and ants, prey upon insects their venom contains large numbers of insecticidal peptide toxins that have evolved to kill or paralyse insect prey. These toxins often modulate the function of their targets with high insect selectivity, lacking any overt toxicity against their vertebrate counterparts (Bende et al., 2013; Gurevitz et al., 2007; Karbat et al., 2004; Wang et al., 2000, 2001; Windley et al., 2011), which can even extend to unique insect family selectivity (Bende et al., 2014). Hence, many of these toxins are being explored as novel insecticides in biopesticide discovery programs (King and Hardy, 2013; Smith et al., 2013; Windley et al., 2012). The limited number of studies on ant venoms would indicate that potential insect-selective peptide neurotoxins are present in their venoms and could be exploited as novel insecticides leads.

Acknowledgements

Financial support for this study was provided by an Australian Postgraduate Award to Samira R. Aili, a Programme Convergence 2007–2013, Région Guyane from the European Community (BI-Appri, 115/SGAR-DE/2011/052274) to Alain Dejean, and a BIOPEPMED grant from the Programme Amazonie II of the French Centre National de la Recherche Scientifique to Pierre Escoubas. This work has also benefited from an “Investissement d’Avenir” grant managed by the Agence Nationale de la Recherche (CEBA, ANR-10-LABX-25-01).

Conflict of interest

The authors declare that there are no conflicts of interest.

Transparency document

Transparency document related to this article can be found online at <http://dx.doi.org/10.1016/j.toxicon.2014.10.021>.

References

- Abbassi, F., Oury, B., Blasco, T., Sereno, D., Bolbach, G., Nicolas, P., Hani, K., Amiche, M., Ladram, A., 2008. Isolation, characterization and molecular cloning of new temporins from the skin of the North African ranid *Pelophylax saharica*. *Peptides* 29, 1526–1533.
- Agosti, D., Johnson, N.F., 2005. Antbase. World Wide Web Electronic Publication antbase.org, version (05/2005). Available online: http://osuc.biosci.ohio-state.edu/hymenoptera/tsa.sppcount?the_taxon=Formicidae (accessed 24.08.14.).
- Altman, R.D., Schultz, D.R., Collins-Yudiskas, B., Aldrich, J., Arnold, P.L., Brown, H.E., 1984. The effects of a partially purified fraction of an ant venom in rheumatoid arthritis. *Arthritis Rheum.* 27, 277–284.
- Amiche, M., Galanth, C., 2011. Dermaseptins as models for the elucidation of membrane-acting helical amphipathic antimicrobial peptides. *Curr. Pharm. Biotechnol.* 12, 1184–1193.
- AntWeb, 2014. Available online: <http://www.antweb.org> (accessed 27.08.14.).
- Argiolas, A., Pisano, J.J., 1985. Bombolitins, a new class of mast cell degranulating peptides from the venom of the bumblebee *Megabombus pennsylvanicus*. *J. Biol. Chem.* 260, 1437–1444.
- Arseniev, A., Pluzhnikov, K., Nolde, D., Sobol, A., Torgov, M.Y., Sukhanov, S., Grishin, E., 1994. Toxic principle of selva ant venom is a pore-forming protein transformer. *FEBS Lett.* 347, 112–116.
- Avitabile, C., Netti, F., Orefice, G., Palmieri, M., Nocerino, N., Malgieri, G., D'Andrea, L.D., Capparelli, R., Fattorusso, R., Romanelli, A., 2013. Design, structural and functional characterization of a temporin-1b analog active against gram-negative bacteria. *Biochim. Biophys. Acta* 1830, 3767–3775.
- Baptista-Saidemberg, N.B., Saidemberg, D.M., Palma, M.S., 2011. Profiling the peptidome of the venom from the social wasp *Agelaia pallipes pallipes*. *J. Proteomics* 74, 2123–2137.
- Barbault, F., Landon, C., Guenneugues, M., Meyer, J.-P., Schott, V., Dimarcq, J.-L., Vovelle, F., 2003. Solution structure of alo-3: a new knottin-type antifungal peptide from the insect *Acrocinus longimanus*. *Biochemistry* 42, 14434–14442.
- Bende, N.S., Dziemborowicz, S., Mobli, M., Herzig, V., Gilchrist, J., Wagner, J., Nicholson, G.M., King, G.F., Bosmans, F., 2014. A distinct sodium channel voltage-sensor locus determines insect selectivity of the spider toxin Dc1a. *Nat. Commun.* 5, 4350.
- Bende, N.S., Kang, E., Herzig, V., Bosmans, F., Nicholson, G.M., Mobli, M., King, G.F., 2013. The insecticidal neurotoxin Aps III is an atypical knottin peptide that potently blocks insect voltage-gated sodium channels. *Biochem. Pharmacol.* 85, 1542–1554.
- Bloch, G., Cohen, M., 2014. The expression and phylogenetics of the inhibitor cysteine knot peptide OCLP1 in the honey bee *Apis mellifera*. *J. Insect Physiol.* 65, 1–8.
- Bosmans, F., Escoubas, P., Nicholson, G.M., 2009. Spider venom peptides as leads for drug and insecticide design. In: de Lima, M.E., Pimenta, A.M.C., Martin-Eauclaire, M.-F., Zingali, R.B., Rochat, H. (Eds.), *Animal Toxins: State of the Art. Perspectives in Health and Biotechnology*. Federal University of Minas Gerais Press, Belo Horizonte, pp. 269–290.
- Bouzid, W., Klopp, C., Verdenaud, M., Ducancel, F., Vetillard, A., 2013. Profiling the venom gland transcriptome of *Tetramorium bicarinatum* (hymenoptera: formicidae): the first transcriptome analysis of an ant species. *Toxicon* 70, 70–81.
- Bradford, A.M., Raftery, M.J., Bowie, J.H., Tyler, M.J., Wallace, J.C., Adams, G.W., Severini, C., 1996. Novel uperin peptides from the dorsal glands of the Australian floodplain toadlet *Uperoleia inundata*. *Aust. J. Chem.* 49, 475–484.
- Brady, S.G., Fisher, B.L., Schultz, T.R., Ward, P.S., 2014. The rise of army ants and their relatives: diversification of specialized predatory doryline ants. *BMC Evol. Biol.* 14, 93–106.
- Brady, S.G., Schultz, T.R., Fisher, B.L., Ward, P.S., 2006. Evaluating alternative hypotheses for the early evolution and diversification of ants. *Proc. Natl. Acad. Sci. U. S. A.* 103, 18172–18177.
- Brand, J.M., 1978. Fire ant venom alkaloids: their contribution to chemosystematics and biochemical evolution. *Biochem. Syst. Ecol.* 6, 337–340.
- Brown, S.G.A., Franks, R.W., Baldo, B.A., Heddle, R.J., 2003. Prevalence, severity, and natural history of jack jumper ant venom allergy in Tasmania. *J. Allergy Clin. Immunol.* 111, 187–192.
- Cammue, B.P., De Bolle, M.F., Terras, F.R., Proost, P., Van Damme, J., Rees, S.B., Vanderleyden, J., Broekaert, W.F., 1992. Isolation and characterization of a novel class of plant antimicrobial peptides from *Mirabilis jalapa* L. seeds. *J. Biol. Chem.* 267, 2228–2233.
- Casewell, N.R., Wuster, W., Vonk, F.J., Harrison, R.A., Fry, B.G., 2013. Complex cocktails: the evolutionary novelty of venoms. *Trends Ecol. Evol.* 28, 219–229.
- Cerdá, X., Dejean, A., 2011. Predation by ants on arthropods and other animals. In: Polidori, C. (Ed.), *Predation in the Hymenoptera: an Evolutionary Perspective*. Transworld Research Network, Kerala - India, pp. 39–78.
- Cologna, C.T., Cardoso J. dos, S., Jourdan, E., Degueldre, M., Upert, G., Gilles, N., Uetanabaro, A.P., Costa Neto, E.M., Thonart, P., de Pauw, E., Quinton, L., 2013. Peptidomic comparison and characterization of the major components of the venom of the giant ant *Dinoponera quadricaps* collected in four different areas of Brazil. *J. Proteomics* 94, 413–422.
- Craik, D.J., Daly, N.L., Waine, C., 2001. The cystine knot motif in toxins and implications for drug design. *Toxicon* 39, 43–60.
- Dai, C., Ma, Y., Zhao, Z., Zhao, R., Wang, Q., Wu, Y., Cao, Z., Li, W., 2008. Mucroporin, the first cationic host defense peptide from the venom of *Lycas mucronatus*. *Antimicrob. Agents Chemother.* 52, 3967–3972.
- Davies, N.W., Wiese, M.D., Brown, S.G., 2004. Characterisation of major peptides in 'jack jumper' ant venom by mass spectrometry. *Toxicon* 43, 173–183.
- de Souza, B.M., Marques, M.R., Tomazela, D.M., Eberlin, M.N., Mendes, M.A., Palma, M.S., 2004. Mass spectrometric characterization of two novel inflammatory peptides from the venom of the social wasp *Polybia paulista*. *Rapid Commun. Mass Spectrom.* 18, 1095–1102.
- Dejean, A., Labrière, N., Touchard, A., Petitclerc, F., Roux, O., 2014. Nesting habits shape feeding preferences and predatory behavior in an ant genus. *Naturwissenschaften* 101, 323–330.
- Dias, N.B., de Souza, B.M., Gomes, P.C., Palma, M.S., 2014. Peptide diversity in the venom of the social wasp *Polybia paulista* (Hymenoptera): a comparison of the intra- and inter-colony compositions. *Peptides* 51, 122–130.
- Donovan, G.R., Baldo, B.A., 1997. In: Pilosulin 2 from Ant Venom, Cloning and Expression of a CDNA Encoding it and its Antihypertensive Properties. PCT International Application, p. 27. WO 9713854.
- Donovan, G.R., Baldo, B.A., Sutherland, S., 1993. Molecular cloning and characterization of a major allergen (Myr p 1) from the venom of the Australian jumper ant, *Myrmecia pilosula*. *Biochim. Biophys. Acta* 1171, 272–280.
- Donovan, G.R., Street, M.D., Baldo, B.A., 1995. Separation of jumper ant (*Myrmecia pilosula*) venom allergens: a novel group of highly basic proteins. *Electrophoresis* 16, 804–810.
- Donovan, G.R., Street, M.D., Baldo, B.A., Alewood, D., Alewood, P., Sutherland, S., 1994. Identification of an IgE-binding determinant of the major allergen Myr p I from the venom of the Australian jumper ant *Myrmecia pilosula*. *Biochim. Biophys. Acta* 1204, 48–52.
- Douglas, R.G., Weiner, J.M., Abramson, M.J., O'Hehir, R.E., 1998. Prevalence of severe ant-venom allergy in southeastern Australia. *J. Allergy Clin. Immunol.* 101, 129–131.
- Duval, A., Malécot, C.O., Pelhate, M., Pieck, T., 1992. Poneratoxin, a new toxin from an ant venom, reveals an interconversion between two gating modes of the Na⁺ channels in frog skeletal muscle fibres. *Pflügers Arch.* 420, 239–247.
- Epad, R.M., Vogel, H.J., 1999. Diversity of antimicrobial peptides and their mechanisms of action. *Biochim. Biophys. Acta* 1462, 11–28.
- Escoubas, P., King, G.F., 2009. Venomics as a drug discovery platform. *Expert Rev. Proteomics* 6, 221–224.
- Fautin, D.G., 2014. Hexacorallians of the World. Available online: <http://geoportal.kgs.ku.edu/hexacoral/anemone2/index.cfm> (accessed 24.08.14.).
- Favreau, P., Menin, L., Michalet, S., Perret, F., Cheneval, O., Stocklin, M., Bulet, P., Stocklin, R., 2006. Mass spectrometry strategies for venom mapping and peptide sequencing from crude venoms: case applications with single arthropod specimen. *Toxicon* 47, 676–687.
- Gilly, W.F., Richmond, T.A., Duda, T.F., Elliger, C., Lebaric, Z., Schulz, J., Bingham, J.P., Sweedler, J.V., 2011. A diverse family of novel peptide toxins from an unusual cone snail, *Conus californicus*. *J. Exp. Biol.* 214, 147–161.
- Gomes, P.C., de Souza, B.M., Dias, N.B., Brigitte, P., Mourelle, D., Arcuri, H.A., dos Santos Cabrera, M.P., Stabeli, R.G., Neto, J.R., Palma, M.S., 2014. Structure-function relationships of the peptide paulistine: a novel toxin from the venom of the social wasp *Polybia paulista*. *Biochim. Biophys. Acta* 1840, 170–183.
- Gurevitz, M., Karbat, I., Cohen, L., Ilan, N., Kahn, R., Turkov, M., Stankiewicz, M., Stühmer, W., Dong, K., Gordon, D., 2007. The insecticidal potential of scorpion β-toxins. *Toxicon* 49, 473–489.
- Haddad Junior, V., Cardoso, J.L., Moraes, R.H., 2005. Description of an injury in a human caused by a false tocadinha (*Dinoponera gigantea*, Perty, 1833) with a revision on folkloric, pharmacological and clinical aspects of the giant ants of the genera *Paraponera* and *Dinoponera* (sub-family Ponerinae). *Rev. Inst. Med. Trop. São Paulo* 47, 235–238.
- Hallan, J., 2005. Synopsis of the Described Scorpiones of the World. Available online: <http://insects.tamu.edu/research/collection/hallan/acari/Scorpiones1.htm> (accessed 24.08.14.).
- Harrison, P.L., Abdel-Rahman, M.A., Miller, K., Strong, P.N., 2014. Antimicrobial peptides from scorpion venoms. *Toxicon* 88C, 115–137.
- Hendrich, A.B., Mozrzymas, J.W., Konopińska, D., Scuka, M., 2001. The effect of poneroatoxin on neuromuscular transmission in the rat diaphragm. *Cell. Mol. Biol. Lett.* 7, 195–202.
- Herzig, V., Wood, D.L., Newell, F., Chaumeil, P.A., Kaas, Q., Binford, G.J., Nicholson, G.M., Gorse, D., King, G.F., 2011. ArachnoServer 2.0, an updated online resource for spider toxin sequences and structures. *Nucleic Acids Res.* 39, D653–D657.
- Hink, W.F., Butz, J.J., 1985. Primary culture of ant venom gland cells. In: *In Vitro Cell Dev Biol* 21, 333–339.
- Hölldobler, B., Wilson, E.O., 1990. *The Ants*. Belknap Press (Harvard University Press), Cambridge, MA.

- Imai, H.T., Taylor, R.W., Crozier, R.H., 1994. Experimental bases for the minimum interaction theory. I. Chromosome evolution in ants of the *Myrmecia pilosula* species complex (hymenoptera: formicidae: myrmeciinae). *Jpn. J. Genet.* 69, 137–182.
- Inagaki, H., Akagi, M., Imai, H.T., Taylor, R.W., Kubo, T., 2004. Molecular cloning and biological characterization of novel antimicrobial peptides, pilosulin 3 and pilosulin 4, from a species of the Australian ant genus *Myrmecia*. *Arch. Biochem. Biophys.* 428, 170–178.
- Inagaki, H., Akagi, M., Imai, H.T., Taylor, R.W., Wiese, M.D., Davies, N.W., Kubo, T., 2008a. Pilosulin 5, a novel histamine-releasing peptide of the Australian ant, *Myrmecia pilosula* (jack jumper ant). *Arch. Biochem. Biophys.* 477, 411–416.
- Inagaki, H., Masuko, K., Kudo, T., 2008b. SKTXs: peptides identified from the ant *Strumigenys kumadori* that block sodium channels. In: 8th-Asia-Pacific Congress on Animal, Plant and Microbial Toxins, Hanoi, Vietnam, p. 75.
- Johnson, B.R., Borowiec, M.L., Chiu, J.C., Lee, E.K., Atallah, J., Ward, P.S., 2013. Phylogenomics resolves evolutionary relationships among ants, bees, and wasps. *Curr. Biol.* 23, 2058–2062.
- Johnson, S.R., Copello, J.A., Evans, M.S., Suarez, A.V., 2010. A biochemical characterization of the major peptides from the venom of the giant neotropical hunting ant *Dinoponera australis*. *Toxicon* 55, 702–710.
- Jones, T., Blum, M., Howard, R., McDaniel, C., Fales, H., DuBois, M., Torres, J., 1982. Venom chemistry of ants in the genus *Monomorium*. *J. Chem. Ecol.* 8, 285–300.
- Jones, T., Torres, J., Spande, T., Garraffo, H., Blum, M., Snelling, R., 1996. Chemistry of venom alkaloids in some *Solenopsis* (diplhoropterum) species from Puerto Rico. *J. Chem. Ecol.* 22, 1221–1236.
- Jones, T., Zottig, V., Robertson, H., Snelling, R., 2003. The venom alkaloids from some African *Monomorium* species. *J. Chem. Ecol.* 29, 2721–2727.
- Jones, T.H., Andersen, A.N., Kenny, J.C., 2009. Venom alkaloid chemistry of Australian species of the *Monomorium rothsteini* complex, with particular reference to taxonomic implications. *Chem. Biodivers.* 6, 1034–1041.
- Jones, T.H., Stahly, S.M., Don, A.W., Blum, M.S., 1988. Chemotaxonomic implications of the venom chemistry of some *Monomorium* “antarcticum” populations. *J. Chem. Ecol.* 14, 2197–2212.
- Karbat, I., Frolow, F., Froy, O., Gilles, N., Cohen, L., Turkov, M., Gordon, D., Gurevitz, M., 2004. Molecular basis of the high insecticidal potency of scorpion α -toxins. *J. Biol. Chem.* 279, 31679–31686.
- Kem, W.R., Wildeboer, K., LeFrancois, S., Raja, M., Marszalec, W., Braekman, J.C., 2004. Nicotinic receptor inhibition by *Tetraponera* ant alkaloids. *Cell. Mol. Neurobiol.* 24, 535–551.
- King, G.F., Hardy, M.C., 2013. Spider-venom peptides: structure, pharmacology, and potential for control of insect pests. *Annu. Rev. Entomol.* 58, 475–496.
- Kohn, A.J., Anderson, T., 2009. The *Conus* Biodiversity Website. Available online: <http://biology.burke.washington.edu/conus/index.php> (accessed 24.08.14.).
- Kuhn-Nentwig, L., 2003. Antimicrobial and cytolytic peptides of venomous arthropods. *Cell. Mol. Life Sci.* 60, 2651–2668.
- Lai, L.C., Kuo, T.C., Huang, R.N., Wu, W.J., 2012. The insecticidal activities of fire ant (hymenoptera: formicidae) venoms against *Plutella xylostella* (lepidoptera: plutellidae) larvae. *J. Econ. Entomol.* 105, 1591–1596.
- Lee, B.J., Seo, M.D., Kang, S.J., Kim, H.J., 2011. Analogues of antimicrobial and anti-cancer peptide synthesized and produced from Gaegurin 5. Promeditech, Inc. (Seoul, KR), Patent 12/301,028.
- Lee, E.J., Lee, J.H., Kim, J.K., Lee, D.G., 2013. Structure-activity relationships of cecropin-like peptides and their interactions with phospholipid membrane. *Biochem. Mol. Biol. Rep.* 46, 284–289.
- Lewis, J.C., Day, A.J., De la Lande, I.S., 1968. Phospholipase A in the venom of the Australian bulldog ant *Myrmecia pyriformis*. *Toxicon* 6, 109–112.
- Lewis, R.J., Garcia, M.L., 2003. Therapeutic potential of venom peptides. *Nat. Rev. Drug Discov.* 2, 790–802.
- Liu, Z., Dai, J., Dai, L., Deng, M., Hu, Z., Hu, W., Liang, S., 2006. Function and solution structure of huwentoxin-X, a specific blocker of N-type calcium channels, from the Chinese bird spider *Ornithoctonus huwena*. *J. Biol. Chem.* 281, 8628–8635.
- Loughnan, M., Nicke, A., Jones, A., Schroeder, C.I., Nevin, S.T., Adams, D.J., Alewood, P.F., Lewis, R.J., 2006. Identification of a novel class of nicotinic receptor antagonists: dimeric conotoxins VxXIIA, VxXIIIB, and VxXIC from *Conus vexillum*. *J. Biol. Chem.* 281, 24745–24755.
- Mackintosh, J., Flood, J., Veal, D., Beattie, A., 1999. Increase in levels of microbiota recoverable from male and larval *Myrmecia gulosa* (fabricius) (hymenoptera: formicidae) following segregation from worker ants. *Aust. J. Entomol.* 38, 124–126.
- Mackintosh, J., Trimble, J., Beattie, A., Veal, D., Jones, M., Karuso, P., 1995. Antimicrobial mode of action of secretions from the metapleural gland of *Myrmecia gulosa* (Australian bull ant). *Can. J. Microbiol.* 41, 136–144.
- Mackintosh, J.A., Veal, D.A., Beattie, A.J., Gooley, A.A., 1998. Isolation from an ant *Myrmecia gulosa* of two inducible O-glycosylated proline-rich antibacterial peptides. *J. Biol. Chem.* 273, 6139–6143.
- Mangoni, M.L., Marcellini, H.G., Simmaco, M., 2007. Biological characterization and modes of action of temporins and bombinin H, multiple forms of short and mildly cationic anti-microbial peptides from amphibian skin. *J. Pept. Sci.* 13, 603–613.
- Mangoni, M.L., Papo, N., Barra, D., Simmaco, M., Bozzi, A., Di Giulio, A., Rinaldi, A.C., 2004. Effects of the antimicrobial peptide temporin L on cell morphology, membrane permeability and viability of *Escherichia coli*. *Biochem. J.* 380, 859–865.
- Maschwitz, U., Hahn, M., Schönenegg, P., 1979. Paralysis of prey in ponerine ants. *Naturwissenschaften* 66, 213–214.
- Maschwitz, U., Jessen, K., Maschwitz, E., 1981. Foaming in *Pachycondyla*: a new defense mechanism in ants. *Behav. Ecol. Sociobiol.* 9, 79–81.
- Matuszek, M.A., Hodgson, W.C., King, R.G., Sutherland, S.K., 1994a. Some enzymic activities of two Australian ant venoms: a jumper ant *Myrmecia pilosula* and a bulldog ant *Myrmecia pyriformis*. *Toxicon* 32, 1543–1549.
- Matuszek, M.A., Hodgson, W.C., Sutherland, S.K., King, R.G., 1992. Pharmacological studies of jumper ant (*Myrmecia pilosula*) venom: evidence for the presence of histamine, and haemolytic and eicosanoid-releasing factors. *Toxicon* 30, 1081–1091.
- Matuszek, M.A., Hodgson, W.C., Sutherland, S.K., King, R.G., 1994b. Pharmacological studies of the venom of an Australian bulldog ant (*Myrmecia pyriformis*). *Nat. Toxins* 2, 36–43.
- McGain, F., Winkel, K.D., 2002. Ant sting mortality in Australia. *Toxicon* 40, 1095–1100.
- McIntosh, J.M., Hasson, A., Spira, M.E., Gray, W.R., Li, W., Marsh, M., Hillyard, D.R., Olivera, B.M., 1995. A new family of conotoxins that blocks voltage-gated sodium channels. *J. Biol. Chem.* 270, 16796–16802.
- Mendes, M.A., de Souza, B.M., Marques, M.R., Palma, M.S., 2004. Structural and biological characterization of two novel peptides from the venom of the neotropical social wasp *Agelaia pallipes pallipes*. *Toxicon* 44, 67–74.
- Moreau, C.S., Bell, C.D., Vila, R., Archibald, S.B., Pierce, N.E., 2006. Phylogeny of the ants: diversification in the age of angiosperms. *Science* 312, 101–104.
- Nicolais, P., Amiche, M., 2013. Dermaseptins. In: Kastin, A.J. (Ed.), *Handbook of Biologically Active Peptides*. Elsevier, Amsterdam, pp. 350–363.
- Nolde, D.E., Sobol, A.G., Pluzhnikov, K.A., Grishin, E.V., Arseniev, A.S., 1995. Three-dimensional structure of ectatommin from *Ectatomma tuberculatum* ant venom. *J. Biomol. NMR* 5, 1–13.
- Norton, R.S., Pallaghy, P.K., 1998. The cystine knot structure of ion channel toxins and related polypeptides. *Toxicon* 36, 1573–1583.
- Orivel, J., 2000. L'adaptation à la vie arboricole de la fourmi *Pachycondyla goeldii* (Hymenoptera: Ponerinae) (PhD Thesis). Université Paris XIII.
- Orivel, J., Dejean, A., 2001. Comparative effect of the venoms of ants of the genus *Pachycondyla* (hymenoptera: ponerinae). *Toxicon* 39, 195–201.
- Orivel, J., Redeker, V., Le Caer, J.P., Krier, F., Revol-Junelles, A.M., Longeon, A., Chaffotte, A., Dejean, A., Rossier, J., 2001. Ponericins, new antibacterial and insecticidal peptides from the venom of the ant *Pachycondyla goeldii*. *J. Biol. Chem.* 276, 17823–17829.
- Ortiz, G., Mathias, M.I., 2006. Venom gland of *Pachycondyla striata* worker ants (hymenoptera: ponerinae). Ultrastructural characterization. *Micron* 37, 243–248.
- Osaki, T., Omotezako, M., Nagayama, R., Hirata, M., Iwanaga, S., Kasahara, J., Hattori, J., Ito, I., Sugiyama, H., Kawabata, S., 1999. Horseshoe crab hemocyte-derived antimicrobial polypeptides, tachystatins, with sequence similarity to spider neurotoxins. *J. Biol. Chem.* 274, 26172–26178.
- Osipov, A.V., Kasheverov, I.E., Makarova, Y.V., Starkov, V.G., Vorontsova, O.V., Ziganshin, R., Andreeva, T.V., Serebryakova, M.V., Benoit, A., Hogg, R.C., Bertrand, D., Tsetlin, V.I., Utkin, Y.N., 2008. Naturally occurring disulfide-bound dimers of three-fingered toxins: a paradigm for biological activity diversification. *J. Biol. Chem.* 283, 14571–14580.
- Pallaghy, P.K., Norton, R.S., Nielsen, K.J., Craik, D.J., 1994. A common structural motif incorporating a cystine knot and a triple-stranded β -sheet in toxic and inhibitory polypeptides. *Protein Sci.* 3, 1833–1839.
- Palma, M.S., 2013. Hymenoptera insect peptides. In: Kastin, A.J. (Ed.), *Handbook of Biologically Active Peptides*. Elsevier, Amsterdam, pp. 416–422.
- Pan, J., Hink, W.F., 2000. Isolation and characterization of myrmexins, six isoforms of venom proteins with anti-inflammatory activity from the tropical ant, *Pseudomyrmex triplarinus*. *Toxicon* 38, 1403–1413.
- Park, C.B., Kim, H.S., Kim, S.C., 1998. Mechanism of action of the antimicrobial peptide buforin II: buforin II kills microorganisms by penetrating the cell membrane and inhibiting cellular functions. *Biochem. Biophys. Res. Commun.* 244, 253–257.
- Piek, T., Duval, A., Hue, B., Karst, H., Lapiel, B., Mantel, P., Nakajima, T., Pelhate, M., Schmidt, J.O., 1991a. Poneratoxin, a novel peptide neurotoxin from the venom of the ant, *Paraponera clavata*. *Comp. Biochem. Physiol. C Comp. Pharmacol. Toxicol.* 99, 487–495.
- Piek, T., Hue, B., Mantel, P., Nakajima, T., Schmidt, J.O., 1991b. Pharmacological characterization and chemical fractionation of the venom of the ponerine ant, *Paraponera clavata* (F.). *Comp. Biochem. Physiol. C Comp. Pharmacol. Toxicol.* 99, 481–486.
- Platnick, N.I., 2014. The World Spider Catalog, Version 14.5. American Museum of Natural History. Available online: <http://research.amnh.org/iz/spiders/catalog> (accessed 24.08.14.).
- Pluzhnikov, K., Nolde, D., Tertyshnikova, S., Sukhanov, S., Sobol, A., Torgov, M., Filippov, A., Arseniev, A., Grishin, E., 1994. Structural and functional studies of toxic principle of *Ectatomma tuberculatum* ant venom. *Bioorg. Khimiia* 20, 857–871 (in Russian).
- Pluzhnikov, K., Nosyreva, E., Shevchenko, L., Kokoz, Y., Schmalz, D., Hucho, F., Grishin, E., 1999. Analysis of ectatommin action on cell membranes. *Eur. J. Biochem.* 262, 501–506.
- Pluzhnikov, K.A., Shevchenko, L.V., Grishin, E.V., 2000. Ant polypeptide toxins. In: Rochat, H., Martin-Eauclaire, M.-F. (Eds.), *Methods and Tools in Biosciences and Medicine: Animal Toxins*. Birkhäuser Verlag, Basel, Switzerland, pp. 90–98.
- Qiu, Y., Choo, Y.M., Yoon, H.J., Jin, B.R., 2012. Molecular cloning and antibacterial activity of bombylolin isolated from the venom of a bumblebee, *Bombus terrestris*. *J. Asia Pacific Entomol.* 15, 21–25.
- Quintero-Hernández, V., Ortiz, E., Rendón-Anaya, M., Schwartz, E.F., Becerril, B., Corzo, G., Possani, L.D., 2011. Scorpion and spider venom peptides: gene cloning and peptide expression. *Toxicon* 58, 644–663.

- Rifflet, A., Gavalda, S., Tene, N., Orivel, J., Leprince, J., Guilhaudis, L., Genin, E., Vettillard, A., Treilhou, M., 2012. Identification and characterization of a novel antimicrobial peptide from the venom of the ant *Tetramorium bicarinatum*. *Peptides* 38, 363–370.
- Rinaldi, A.C., Conlon, J.M., 2013. Temporins. In: Kastin, A.J. (Ed.), *Handbook of Biologically Active Peptides*. Elsevier, Amsterdam, pp. 400–406.
- Rodríguez, A.A., Salceda, E., Garateix, A.G., Zaharenko, A.J., Peigneur, S., López, O., Pons, T., Richardson, M., Díaz, M., Hernández, Y., Ständer, L., Tytgat, J., Soto, E., 2014. A novel sea anemone peptide that inhibits acid-sensing ion channels. *Peptides* 53, 3–12.
- Rykačewska-Czerwinska, M., Radosz, A., Konopinska, D., Wrobel, M., Plech, A., 2008. Antinociceptive effect of poneratoxin (PoTX) in rats. *Pesticydy 1-2*, 135–141.
- Santos, A.D., Imperial, J.S., Chaudhary, T., Beavis, R.C., Chait, B.T., Hunsperger, J.P., Olivera, B.M., Adams, M.E., Hillyard, D.R., 1992. Heterodimeric structure of the spider toxin ω -agatoxin IA revealed by precursor analysis and mass spectrometry. *J. Biol. Chem.* 267, 20701–20705.
- Sarray, S., Luis, J., Ayeb, M.E., Marrakchi, N., 2013. Snake venom peptides: promising molecules with anti-tumor effects. In: Hernández-Ledesma, B., Hsieh, C.-C. (Eds.), *Bioactive Food Peptides in Health and Disease*. InTech, Croatia, pp. 219–238.
- Schmidt, C.A., Shattuck, S.O., 2014. The higher classification of the ant subfamily Ponerinae (Hymenoptera: Formicidae), with a review of ponerine ecology and behavior. *Zootaxa* 3817, 1–242.
- Schmidt, J.O., 1982. Biochemistry of insect venoms. *Annu. Rev. Entomol.* 27, 339–368.
- Schmidt, J.O., 1990. Hymenopteran venoms: striving toward the ultimate defense against vertebrates. In: Evans, D.L., Schmidt, J.O. (Eds.), *Insect Defenses: Adaptive Mechanisms and Strategies of Prey and Predators*. State University of New York Press, Albany, pp. 387–419.
- Schmidt, J.O., 2014. Evolutionary responses of solitary and social hymenoptera to predation by primates and overwhelmingly powerful vertebrate predators. *J. Hum. Evol.* 71, 12–19.
- Schmidt, J.O., Blum, M.S., 1978a. A harvester ant venom: chemistry and pharmacology. *Science* 200, 1064–1066.
- Schmidt, J.O., Blum, M.S., 1978b. Pharmacological and toxicological properties of harvester ant, *Pogonomyrmex badius*, venom. *Toxicon* 16, 645–651.
- Schmidt, J.O., Blum, M.S., Overal, W.L., 1983. Hemolytic activities of stinging insect venoms. *Arch. Insect Biochem. Physiol.* 1, 155–160.
- Schmidt, J.O., Snelling, G.C., 2009. *Pogonomyrmex anzensis* cole: does an unusual harvester ant species have an unusual venom? *J. Hym. Res.* 18, 322–325.
- Schultz, D.R., Arnold, P.I., 1984. Immunochemical and clinical studies of venom from the ant *Pseudomyrmex* sp. In: Tu, A.T. (Ed.), *Handbook of Natural Toxicon, Insect Poisons, Allergens and Other Invertebrate Venoms*. Marcel Dekker, New York, pp. 243–290.
- Simmaco, M., Mignogna, G., Canofeni, S., Miele, R., Mangoni, M.L., Barra, D., 1996. Temporins, antimicrobial peptides from the European red frog *Rana temporaria*. *Eur. J. Biochem.* 242, 788–792.
- Smith, J.J., Herzig, V., King, G.F., Alewood, P.F., 2013. The insecticidal potential of venom peptides. *Cell. Mol. Life Sci.* 70, 3665–3693.
- Starr, C.K., 1985. A simple pain scale for field comparison of hymenopteran stings. *J. Entomol. S. C.* 20, 225–231.
- Steinborner, S.T., Bowie, J.H., Tyler, M.J., Wallace, J.C., 1997. An unusual combination of peptides from the skin glands of Ewing's tree frog, *Litoria ewingi*. Sequence determination and antimicrobial activity. *Aust. J. Chem.* 50, 889–894.
- Street, M.D., Donovan, G.R., Baldo, B.A., 1996. Molecular cloning and characterization of the major allergen *Myr p II* from the venom of the jumper ant *Myrmecia pilosula*: *Myr p I* and *Myr p II* share a common protein leader sequence. *Biochim. Biophys. Acta* 1305, 87–97.
- Street, M.D., Donovan, G.R., Baldo, B.A., Sutherland, S., 1994. Immediate allergic reactions to *Myrmecia* ant stings: immunochemical analysis of *Myrmecia* venoms. *Clin. Exp. Allergy* 24, 590–597.
- Suzuki, H., Iwamuro, S., Ohnuma, A., Coquet, L., Leprince, J., Jouenne, T., Vaudry, H., Taylor, C.K., Abel, P.W., Conlon, J.M., 2007. Expression of genes encoding antimicrobial and bradykinin-related peptides in skin of the stream brown frog *Rana sakuraii*. *Peptides* 28, 505–514.
- Szolajka, E., Poznanski, J., Ferber, M.L., Michalik, J., Gout, E., Fender, P., Baily, I., Dublet, B., Chroboczek, J., 2004. Poneratoxin, a neurotoxin from ant venom. Structure and expression in insect cells and construction of a bio-insecticide. *Eur. J. Biochem.* 271, 2127–2136.
- Téne, N., Roche-Chatain, V., Rifflet, A., Bonnafé, E., Lefranc, B., Leprince, J., Treilhou, M., 2014. Potent bactericidal effects of bicarinatin against strains of the *Enterobacter* and *Cronobacter* genera. *Food Control* 42, 202–206.
- Torres, A.F.C., Huang, C., Chong, C.-M., Leung, S.W., Prieto-da-Silva, A.R.B., Hvat, A., Quinet, Y.P., Martins, A.M.C., Lee, S.M.Y., Rádis-Baptista, G., 2014. Transcriptome analysis in venom gland of the predatory giant ant *Dinoponera quadriceps*: insights into the polypeptide toxin arsenal of Hymenopterans. *PLoS One* 9, e87556.
- Touchard, A., Dauvois, M., Arguel, M.J., Petitclerc, F., Leblanc, M., Dejean, A., Orivel, J., Nicholson, G.M., Escoubas, P., 2014a. Elucidation of the unexplored biodiversity of ant venom peptidomes via MALDI-TOF mass spectrometry and its application for chemotaxonomy. *J. Proteomics* 105, 217–231.
- Touchard, A., Labrière, N., Roux, O., Petitclerc, F., Orivel, J., Escoubas, P., Koh, J.M., Nicholson, G.M., Dejean, A., 2014b. Venom toxicity and composition in three *Pseudomyrmex* ant species having different nesting modes. *Toxicon* 88, 67–76.
- Uetz, P., Hošek, J., 2014. The Reptile Database. Available online: <http://www.reptiledatabase.org> (accessed 24.08.14).
- van Emden, H., 2013. Subclass pterygota, division endopterygota, order hymenoptera (sawflies, ants, bees and wasps) – c. 120,000 described species. In: *Handbook of Agricultural Entomology*. John Wiley & Sons, pp. 193–220.
- Vetter, I., Davis, J.L., Rash, L.D., Anangi, R., Mobli, M., Alewood, P.F., Lewis, R.J., King, G.F., 2011. Venomics: a new paradigm for natural products-based drug discovery. *Amino Acids* 40, 15–28.
- Vetter, I., Lewis, R.J., 2012. Therapeutic potential of cone snail venom peptides (conopeptides). *Curr. Top. Med. Chem.*
- Viljakainen, L., Pamilo, P., 2008. Selection on an antimicrobial peptide defensin in ants. *J. Mol. Evol.* 67, 643–652.
- Vizzavona, J., Zufferey, A., Rose, K., 2009. Synthesis and characterization of dimeric venom peptides. *Adv. Exp. Med. Biol.* 611, 493–494.
- von Sicard, N.A., Candy, D.J., Anderson, M., 1989. The biochemical composition of venom from the pavement ant (*Tetramorium caespitum* L.). *Toxicon* 27, 1127–1133.
- Wang, X., Connor, M., Smith, R., Maciejewski, M.W., Howden, M.E., Nicholson, G.M., Christie, M.J., King, G.F., 2000. Discovery and characterization of a family of insecticidal neurotoxins with a rare vicinal disulfide bridge. *Nat. Struct. Biol.* 7, 505–513.
- Wang, X.H., Connor, M., Wilson, D., Wilson, H.I., Nicholson, G.M., Smith, R., Shaw, D., Mackay, J.P., Alewood, P.F., Christie, M.J., King, G.F., 2001. Discovery and structure of a potent and highly specific blocker of insect calcium channels. *J. Biol. Chem.* 276, 40306–40312.
- Wiese, M.D., Brown, S.G., Chataway, T.K., Davies, N.W., Milne, R.W., Aulfrey, S.J., Heddle, R.J., 2007. *Myrmecia pilosula* (jack jumper) ant venom: identification of allergens and revised nomenclature. *Allergy* 62, 437–443.
- Wiese, M.D., Chataway, T.K., Davies, N.W., Milne, R.W., Brown, S.G., Gai, W.P., Heddle, R.J., 2006. Proteomic analysis of *Myrmecia pilosula* (jack jumper) ant venom. *Toxicon* 47, 208–217.
- Wilson, E.O., 1971. *The Insect Societies*. Harvard University Press, Cambridge, MA.
- Wilson, E.O., 1990. Success and Dominance in Ecosystems: the Case of the Social Insects. Ecology Institute, Oldendorf/Luhe, Germany.
- Windley, M.J., Escoubas, P., Valenzuela, S.M., Nicholson, G.M., 2011. A novel family of insect-selective peptide neurotoxins targeting insect large-conductance calcium-activated K^+ channels isolated from the venom of the theraphosid spider *Eucratoscelus constrictus*. *Mol. Pharmacol.* 80, 1–13.
- Windley, M.J., Herzig, V., Dziemborowicz, S.A., Hardy, M.C., King, G.F., Nicholson, G.M., 2012. Spider-venom peptides as bioinsecticides. *Toxins* 4, 191–227.
- Wu, Q.X., King, M.A., Donovan, G.R., Alewood, P., Sawyer, W.H., Baldo, B.A., 1998. Cytotoxicity of pilosulin 1, a peptide from the venom of the jumper ant *Myrmecia pilosula*. *Biochim. Biophys. Acta* 1425, 74–80.
- Yek, S.H., Mueller, U.G., 2011. The metapleural gland of ants. *Biol. Rev. Camb. Philos. Soc.* 86, 774–791.
- Yi, G.B., McClendon, D., Desaiah, D., Goddard, J., Lister, A., Moffitt, J., Meer, R.K., deShazo, R., Lee, K.S., Rockhold, R.W., 2003. Fire ant venom alkaloid, iso-solenopsin A, a potent and selective inhibitor of neuronal nitric oxide synthase. *Int. J. Toxicol.* 22, 81–86.
- Zamudio, F.Z., Conde, R., Arevalo, C., Becerril, B., Martin, B.M., Valdivia, H.H., Possani, L.D., 1997. The mechanism of inhibition of ryanodine receptor channels by imperatoxin I, a heterodimeric protein from the scorpion *Pandinus imperator*. *J. Biol. Chem.* 272, 11886–11894.
- Zelezetsky, I., Pag, U., Antcheva, N., Sahl, H.G., Tossi, A., 2005. Identification and optimization of an antimicrobial peptide from the ant venom toxin pilosulin. *Arch. Biochem. Biophys.* 434, 358–364.
- Zeng, X.C., Wang, S.X., Zhu, Y., Zhu, S.Y., Li, W.X., 2004. Identification and functional characterization of novel scorpion venom peptides with no disulfide bridge from *Buthos martensi* Karsch. *Peptides* 25, 143–150.
- Zhang, Z., Zhu, S., 2012. Comparative genomics analysis of five families of antimicrobial peptide-like genes in seven ant species. *Dev. Comp. Immunol.* 38, 262–274.
- Zhu, S., Darbon, H., Dyason, K., Verdonck, F., Tytgat, J., 2003. Evolutionary origin of inhibitor cystine knot peptides. *FASEB J.* 17, 1765–1767.